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## (54) NOVEL OSTEOINDUCTIVE COMPOSITIONS

OSTEOINDUKTIVE MITTEL

**NOUVELLES COMPOSITIONS OSTEOINDUCTIVES** 

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#### Description

The present invention relates to novel proteins, processes for obtaining them and genes encoding them. These proteins are capable of inducing cartilage and bone formation.

#### Background

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Bone is a highly specialized tissue characterized by an extensive matrix structure formed of fibrous bundles of the protein collagen, and proteoglycans, noncollagenous proteins, lipids and acidic proteins. The processes of bone formation and renewal/repair of bone tissue, which occur continuously throughout life, are performed by specialized cells. Normal embryonic long bone development is preceded by formation of a cartilage model. Bone growth is presumably mediated by "osteoblasts" (bone-forming cells), while remodeling of bone is apparently accomplished by the joint activities of bone-resorbing cells, called "osteoclasts" and osteoblasts. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g. European patent applications 148,155 and 169,016 for discussions thereof.

#### Brief Description of the Invention

The present invention provides novel proteins in purified form and genes encoding them. Specifically, two of the novel proteins are designated BMP-2 Class I (or BMP-2), and BMP-2 Class II (or BMP-4) wherein BMP is bone morphogenic protein. These proteins are characterized by peptide sequences the same as or substantially homologous to amino acid sequences illustrated in Tables II, III and IV below. They are capable of inducing bone formation at a predetermined site. These bone inductive factors are further characterized by biochemical and biological characteristics including activity at a concentration of 10 to 1000ng/gram of bone in an in vivo rat bone formation assay described below. Proteins of this invention may be encoded by the DNA sequences depicted in the Tables or by sequences capable of hybridizing thereto and coding for polypeptides with bone growth factor biological properties or other variously modified sequences demonstrating such properties.

One of the proteins of the invention is designated BMP-2 Class I (or BMP-2). It is characterized by at least a portion of a peptide sequence the same or substantially the same as that of amino acid #1 through amino acid #396 of Table III which represents the cDNA hBMP-2 Class I. This peptide sequence is encoded by the same or substantially the same DNA sequence, as depicted in nucleotide #356 through nucleotide #1543 of Table III. The human peptide sequence identified in Table III is 396 amino acids in length. hBMP-2 or related bone inductive proteins may also be characterized by at least a portion of this peptide sequence. hBMP-2 Class I is further characterized by the ability to induce bone formation.

The homologous bovine bone inductive protein of the invention designated bBMP-2 Class I (or bBMP-2), has a DNA sequence identified in Table II below which represents the genomic sequence. This bovine DNA sequence has a prospective 129 amino acid coding sequence followed by approximately 205 nucleotides (a presumptive 3' non-coding sequence). bBMP-2, Class I is further characterized by the ability to induce bone formation. A further bone inductive protein composition of the invention is designated BMP-2 Class II or BMP-4. The human protein hBMP-2 Class II (or hBMP-4) is characterized by at least a portion of the same or substantially the same peptide sequence between amido acid #1 through amino acid #408 of Table IV, which represents the cDNA of hBMP-2 Class II. This peptide sequence is encoded by at least a portion of the same or substantially the same DNA sequence as depicted in nucleotide #403 through nucleotide #1626 of Table IV. This factor is further characterized by the ability to induce bone formation.

Another aspect of the invention provides pharmaceutical compositions containing a therapeutically effective amount of one or more bone growth factor polypeptides according to the invention in a pharmaceutically acceptable vehicle. These compositions may further include other therapeutically useful agents. They may also include an appropriate matrix for delivering the proteins to the site of the bone defect and for providing a structure for bone growth. These compositions may be employed in methods for treating a number of bone defects and periodontal disease. These methods, according to the invention, entail administering to a patient needing such bone formation an effective amount of at least one of the novel proteins BMP-2 Class I and BMP-2 Class-II as described herein.

Still a further aspect of the invention are DNA sequences coding on expression for a human or bovine polypeptide having the ability to induce bone formation. Such sequences include the sequence of nucleotides in a 5' to 3' direction illustrated in Tables II, III and IV. Alternatively, a DNA sequence which hybridizes under stringent conditions with the DNA sequences of Tables II, III and IV or a DNA sequence which hybridizes under non-stringent conditions with the illustrated DNA sequences and which codes on expression for a protein having at least one bone growth factor biological property are included in the present invention. Finally, allelic or other variations of the sequences of Tables II, III and IV, whether such nucleotide changes result in changes in the peptide sequence or not, are also included in the present

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Still a further aspect of the invention is a vector containing a DNA sequence as described above in operative association with an expression control sequence. Such vector may be employed in a novel process for producing a bone growth factor polypeptide in which a cell line transformed with a DNA sequence encoding expression of a bone growth factor polypeptide in operative association with an expression control sequence therefor, is cultured. This claimed process may employ a number of known cells as host cells for expression of the polypeptide. Presently preferred cell lines are mammalian cell lines and bacterial cells.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

**Detailed Description of the Invention** 

The proteins of the present invention are characterized by amino acid sequences or portions thereof the same as or substantially homologous to the sequences shown in Tables II, III and IV. These proteins are also characterized by the ability to induce bone formation.

The bone growth factors provided herein also include factors encoded by the sequences similar to those of Tables II, III and IV, but into which modifications are naturally provided (e.g. allelic variations in the nucleotide sequence which may result in amino acid changes in the polypeptide) or deliberately engineered. For example, synthetic polypeptides may wholly or partially duplicate continuous sequences of the amino acid residues of Tables II, III and IV. These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with bone growth factor polypeptides of Tables II, III and IV may possess bone growth factor biological properties in common therewith. Thus, they may be employed as biologically active substitutes for naturally-occurring bone growth factor polypeptides in therapeutic processes.

Other specific mutations of the sequences of the bone growth factors described herein involve modifications of one or both of the glycosylation sites. The absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at one or both of the asparagine-linked glycosylation recognition sites present in the sequences of the bone growth factors shown in Tables II, III and IV. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second postion) results in non-glycosylation at the modified tripeptide sequence.

The present invention also encompasses the novel DNA sequences, free of association with DNA sequences encoding other proteinaceous materials, and coding on expression for bone growth factors. These DNA sequences include those depicted in Tables II, III and IV in a 5' to 3' direction and those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences of Tables II, III and IV.

DNA sequences which hybridize to the sequences of Tables II, III and IV under relaxed hybridization conditions and which code on expression for bone growth factors having bone growth factor biological properties also encode bone growth factors of the invention. For example, a DNA sequence which shares regions of significant homology, e. g., sites of glycosylation or disulfide linkages, with the sequences of Tables II, III and IV and encodes a bone growth factor having one or more bone growth factor biological properties clearly encodes a member of this novel family of growth factors, even if such a DNA sequence would not stringently hybridize to the sequence of Tables II, III and IV.

Similarly, DNA sequences which code for bone growth factor polypeptides coded for by the sequences of Tables II, III and IV, but which differ in codon sequence due to the degeneracies of the genetic code or allelic variations (naturally-occurring base changes in the species population which may or may not result in an amino acid change) also encode the novel growth factors described herein. Variations in the DNA sequences of Tables II, III and IV which are caused by point mutations or by induced modifications to enhance the activity, half-life or production of the polypeptides encoded thereby are also encompassed in the invention.

Another aspect of the present invention provides a novel method for producing the novel osteoinductive factors. The method of the present invention involves culturing a suitable cell or cell line, which has been transformed with a DNA sequence coding on expression for a novel bone growth factor polypeptide of the invention, under the control of known regulatory sequences. Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary (CHo) cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293: 620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U.S. Patent 4,419,446. Another suitable mammalian cell line, which is described in the accompanying examples, is the monkey COS-1 cell line. A similarly useful mammalian cell line is the CV-1 cell line.

Bacterial cells are suitable hosts. For example, the various strains of E. <u>coli</u> (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of <u>B</u>. <u>subtilis</u>, <u>Pseudomonas</u>, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, <u>Genetic Engineering</u>, <u>8</u>:277-298 (Plenum Press 1986) and references cited therein.

Another aspect of the present invention provides vectors for use in the method of expression of these novel osteoinductive polypeptides. Preferably the vectors contain the full novel DNA sequences described above which code for
the novel factors of the invention. Additionally the vectors also contain appropriate expression control sequences permitting expression of the bone inductive protein sequences. Alternatively, vectors incorporating modified sequences
as described above are also embodiments of the present invention and useful in the production of the bone inductive
proteins. The vectors may be employed in the method of transforming cell lines and contain selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the
replication and expression thereof in selected host cells. Useful regulatory sequences for such vectors are known to
one of skill in the art and may be selected depending upon the selected host cells. Such selection is routine and does
not form part of the present invention.

A protein of the present invention, which induces bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures. An osteogenic preparation employing one or more of the proteins of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery. An osteogenic factor of the invention may be valuable in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. Of course, the proteins of the invention may have other therapeutic uses.

A further aspect of the invention is a therapeutic method and composition for repairing fractures and other conditions related to bone defects or periodontal diseases. Such a composition comprises a therapeutically effective amount of at least one of the bone inductive factor proteins of the invention. The bone inductive factors according to the present invention may be present in a therapeutic composition in admixture with a pharmaceutically acceptable vehicle or matrix. Further therapeutic methods and compositions of the invention comprise a therapeutic amount of a bone inductive factor of the invention with a therapeutic amount of at least one of the other bone inductive factors of the invention. Additionally, the proteins according to the present invention or a combination of the proteins of the present invention may be co-administered with one or more different osteoinductive factors with which they may interact. Further, the bone inductive proteins may be combined with other agents beneficial to the treatment of the bone defect in question. Such agents include, but are not limited to various growth factors. The preparation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

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In particular, BMP-2 Class I may be used individually in a pharmaceutical composition. BMP-2 Class I may also be used in combination with one or more of the other proteins of the invention. BMP-2 Class I may be combined with BMP-2 Class II. It may also be combined with BMP-3. Further BMP-2 Class I may be combined with BMP-2 Class II and BMP-3.

BMP-2 Class II may be used individually in pharmaceutical composition. In addition, it may be used in combination with other proteins as identified above. Further it may be used in combination with BMP-3.

The therapeutic method includes locally administering the composition as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone damage. Preferably, the bone growth inductive factor composition would include a matrix capable of delivering the bone inductive factor to the site of bone damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of other materials presently in use for other implanted medical applications.

The choice of material is based on, for example, biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. Similarly, the application of the osteoinductive factors will define the appropriate formulation. Potential matrices for the osteoinductive factors may be biodegradable and chemically defined, such as, but not limited to calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyanhydrides; biodegradable and biologically well defined, such as bone or dermal collagen, other pure proteins or extracellular matrix components; nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics; or combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics might also be altered in composition, such as in calcium-aluminate-phos-

phate and processing to alter for example, pore size, particle size, particle shape, and biodegradability.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of such a growth factor, e.g. amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the composition of BMP's. The addition of other known growth factors, such as IGF 1 (insulin like growth factor 1), to the final composition, may also effect the dosage. Generally, the dosage regimen should be in the range of approximately 10 to 10<sup>6</sup> nanograms of protein per gram of bone weight desired. Progress can be monitored by periodic assessment of bone growth and/ or repair, e.g. x-rays. Such therapeutic compositions are also presently valuable for veterinary applications due to the lack of species specificity in bone inductive factors. Particularly domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with the bone inductive factors of the present invention.

The following examples illustrate practice of the present invention in recovering and characterizing the bovine proteins and employing them to recover the human proteins, obtaining the human proteins and in expressing the proteins via recombinant techniques.

**EXAMPLE I** 

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#### Isolation of Bovine Bone Inductive Factor

Ground bovine bone powder (20-120 mesh, Helitrex) is prepared according to the procedures of M. R. Urist et al., Proc. Natl Acad. Sci USA, 70:3511 (1973) with elimination of some extraction steps as identified below. Ten kgs of the ground powder is demineralized in succesive changes of 0.6N HCl at 4°C over a 48 hour period with vigorous stirring. The resulting suspension is extracted for 16 hours at 4°C with 50 liters of 2M CaCl<sub>2</sub> and 10mM ethylenediaminetetraacetic acid [EDTA], and followed by extraction for 4 hours in 50 liters of 0.5M EDTA. The residue is washed three times with distilled water before its resuspension in 20 liters of 4M guanidine hydrochloride [GuCl], 20mM Tris (pH 7.4), 1 mM N-ethylmaleimide, 1mM iodoacetamide, 1mM phenylmethylsulfonyl fluoride as described in Clin. Orthop. Rel. Res., 171: 213 (1982). After 16 to 20 hours the supernatant is removed and replaced with another 10 liters of GuCl buffer. The residue is extracted for another 24 hours.

The crude GuCl extracts are combined, concentrated approximately 20 times on a Pellicon apparatus with a 10,000 molecular weight cut-off membrane, and then dialyzed in 50mM Tris, 0.1M NaCl, 6M urea (pH7.2), the starting buffer for the first column. After extensive dialysis the protein is loaded on a 4 liter DEAE cellulose column and the unbound fractions are collected.

The unbound fractions are concentrated and dialyzed against 50mM NaAc, 50mM NaCl (pH 4.6) in 6M urea. The unbound fractions are applied to a carboxymethyl cellulose column. Protein not bound to the column is removed by extensive washing with starting buffer, and the bone inductive factor containing material desorbed from the column by 50mM NaAc, 0.25mM NaCl, 6M urea (pH 4.6). The protein from this step elution is concentrated 20- to 40- fold, then diluted 5 times with 80mM KPO<sub>4</sub>, 6M urea (pH6.0). The pH of the solution is adjusted to 6.0 with 500mM K<sub>2</sub>HPO<sub>4</sub>. The sample is applied to an hydroxylapatite column (LKB) equilibrated in 80mM KPO<sub>4</sub>, 6M urea (pH6.0) and all unbound protein is removed by washing the column with the same buffer. Bone inductive factor activity is eluted with 100mM KPO<sub>4</sub> (pH7.4) and 6M urea.

The protein is concentrated approximately 10 times, and solid NaCl added to a final concentration of 0.15M. This material is applied to a heparin - Sepharose column equilibrated in 50mM KPO<sub>4</sub>, 150mM NaCl, 6M urea (pH7.4). After extensive washing of the column with starting buffer, a protein with bone inductive factor activity is eluted by 50mM KPO<sub>4</sub>, 700mM NaCl, 6M urea (pH7.4). This fraction is concentrated to a minimum volume, and 0.4ml aliquots are applied to Superose 6 and Superose 12 columns connected in series, equilibrated with 4M GuCl, 20mM Tris (pH7.2) and the columns developed at a flow rate of 0.25ml/min. The protein demonstrating bone inductive factor activity has a relative migration corresponding to approximately 30,000 dalton protein.

The above fractions are pooled, dialyzed against 50mM NaAc, 6M urea (pH4.6), and applied to a Pharmacia MonoS HR column. The column is developed with a gradient to 1.0M NaCl, 50mM NaAc, 6M urea (pH4.6). Active fractions are pooled and brought to pH3.0 with 10% trifluoroacetic acid (TFA). The material is applied to a 0.46 x 25cm Vydac C4 column in 0.1% TFA and the column developed with a gradient to 90% acetonitrile, 0.1% TFA (31.5% acetonitrile, 0.1% TFA to 49.5% acetonitrile, 0.1% TFA in 60 minutes at Iml per minute). Active material is eluted at approximately 40-44% acetonitrile. Aliquots of the appropriate fractions are iodinated by one of the following methods: P. J. McConahey et al, Int. Arch. Allergy, 29:185-189 (1966); A. E. Bolton et al, Biochem J., 133:529 (1973); and D. F. Bowen-Pope, J. Biol. Chem., 237:5161 (1982). The iodinated proteins present in these fractions are analyzed by SDS gel electrophoresis and urea Triton X 100 isoelectric focusing. At this stage, the bone inductive factor is estimated to be approximately 10-50% pure.

#### **EXAMPLE II**

#### Characterization of Bovine Bone Inductive Factor

#### A. Molecular Weight

Approximately 20ug protein from Example I is lyophilized and redissolved in 1X SDS sample buffer. After 15 minutes of heating at 37°C, the sample is applied to a 15% SDS polyacrylamide gel and then electrophoresed with cooling. The molecular weight is determined relative to prestained molecular weight standards (Bethesda Research Labs). Immediately after completion, the gel lane containing bone inductive factor is sliced into 0.3cm pieces. Each piece is mashed and 1.4ml of 0.1% SDS is added. The samples are shaken gently overnight at room temperature to elute the protein. Each gel slice is desalted to prevent interference in the biological assay. The supernatant from each sample is acidified to pH 3.0 with 10% TFA, filtered through a 0.45 micron membrane and loaded on a 0.46cm x 5cm C4 Vydac column developed with a gradient of 0.1% TFA to 0.1% TFA, 90% CH<sub>3</sub>CN. The appropriate bone inductive factor containing fractions are pooled and reconstituted with 20mg rat matrix. In this gel system, the majority of bone inductive factor fractions have the mobility of a protein having a molecular weight of approximately 28,000 - 30,000 daltons.

#### B. Isoelectric Focusing

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The isoelectric point of bone inductive factor activity is determined in a denaturing isoelectric focusing system. The Triton X100 urea gel system (Hoeffer Scientific) is modified as follows: 1) 40% of the ampholytes used are Servalyte 3/10; 60% are Servalyte 7-9. 2) The catholyte used is 40mM NaOH. Approximately 20ug of protein from Example I is lyophilized, dissolved in sample buffer and applied to the isoelectrofocusing gel. The gel is run at 20 watts, 10°C for approximately 3 hours. At completion the lane containing bone inductive factor is sliced into 0.5 cm slices. Each piece is mashed in 1.0ml 6M urea, 5mM Tris (pH 7.8) and the samples agitated at room temperature. The samples are acidified, filtered, desalted and assayed as described above. The major portion of activity as determined in the assay described in Example III migrates in a manner consistent with a pl of 8.8 - 9.2.

#### C. Subunit Characterization

The subunit composition of bone inductive factor is also determined. Pure bone inductive factor is isolated from a preparative 15% SDS gel as described above. A portion of the sample is then reduced with 5mM DTT in sample buffer and re-electrophoresed on a 15% SDS gel. The approximately 30kd protein yields two major bands at approximately 20kd and 18kd, as well as a minor band at 30kd. The broadness of the two bands indicates heterogeneity caused most probably by glycosylation, other post translational modification, proteolytic degradation or carbamylation.

#### **EXAMPLE III**

#### Biological Activity of Bone Inductive Factor

A rat bone formation assay according to the general procedure of Sampath and Reddi, <u>Proc. Natl. Acad. Sci. U. S.A.</u>, 80:6591-6595 (1983) is used to evaluate the osteogenic activity of the bovine bone inductive factor of the present invention obtained in Example I. This assay can also be used to evaluate bone inductive factors of other species. The ethanol precipitation step is replaced by dialyzing the fraction to be assayed against water. The solution or suspension is then redissolved in a volatile solvent, e.g. 0.1 - 0.2 % TFA, and the resulting solution added to 20mg of rat matrix. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., <u>Proc. Natl. Acad. Sci.</u>, 69:1601 (1972)] and half is fixed and processed for histological analysis. Routinely, 1µm glycolmethacrylate sections are stained with Von Kossa and acid fuchsin to detect new bone mineral. Alkaline phosphatase, an enzyme produced by chondroblasts and osteoblasts in the process of matrix formation, is also measured. New cartilage and bone formation often correlates with alkaline phosphatase levels. Table I below illustrates the dose response of the rat matrix samples including a control not treated with bone inductive factor.

TABLE 1

Protein* Implanted μg	Cartilage	Alk. Phos.u/l
7.5	2	Not done
2.5	3	445.7
0.83	3	77.4
0.28	0	32.5
0.00	0	31.0

\*At this stage the bone inductive factor is approximately 10-15% pure.

The bone or cartilage formed is physically confined to the space occupied by the matrix. Samples are also analyzed by SDS gel electrophoresis and isoelectric focusing as described above, followed by autoradiography. Analysis reveals a correlation of activity with protein bands at 28 - 30kd and a pl 9.0. An extinction coefficient of 1 OD/mg-cm is used as an estimate for protein and approximating the purity of bone inductive factor in a particular fraction. In the <u>in vivo</u> rat bone formation assays on dilutions as described above, the protein is active <u>in vivo</u> at 10 to 200ng protein/gram bone to probably greater than 1µg protein/gram bone.

## **EXAMPLE IV**

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#### Bovine Bone Inductive Factor Protein Composition

The protein composition of Example IIA of molecular weight 28 - 30kd is reduced as described in Example IIC and digested with trypsin. Eight tryptic fragments are isolated by standard procedures having the following amino acid sequences:

Fragment 1: A A F L G D I A L D E E D L G

Fragment 2: A F Q V Q Q A A D L

Fragment 3: N Y Q D M V V E G

Fragment 4: STPAQDVSR

Fragment 5: NQEALR

Fragment 6: LSEPDPSHTLEE

Fragment 7: F D A Y Y

Fragment 8: LKPSN?ATIQSIVE

A less highly purified preparation of protein from bovine bone is prepared according to a purification scheme similar to that described in Example I. The purification basically varies from that previously described by omission of the DE-52 column, the CM cellulose column and the mono s column, as well as a reversal in the order of the hydroxylapatite and heparin sepharose columns. Briefly, the concentrated crude 4 M extract is brought to 85% final concentration of ethanol at 4 degrees. The mixture is then centrifuged, and the precipitate redissolved in 50 mM Tris, 0.15 M NaCl, 6.0 M urea. This material is then fractionated on Heparin Sepharose as described. The Heparin bound material is fractionated on hydroxyapatite as described. The active fractions are pooled, concentrated, and fractionated on a high resolution gel filtration (TSK 30000 in 6 M guanidinium chloride, 50 mM Tris, pH 7.2). The active fractions are pooled, dialyzed against 0.1% TFA, and then fractionated on a C4 Vydac reverse phase column as described. The preparation is reduced and electrophoresed on an acrylamide gel. The protein corresponding to the 18K band is eluted and digested with trypsin. Tryptic fragments are isolated having the following amino acid sequences:

Fragment 9: SLKPSNHATIQS? V

Fragment 10: S F D A Y Y C S ? A

Fragment 11: V Y P N M T V E S C A

Fragment 12: V D F A D I ? W

Tryptic Fragments 7 and 8 are noted to be substantially the same as Fragments 10 and 9, respectively.

#### A. bBMF-2

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Two probes consisting of pools of oligonucleotides are designed on the basis of the amino acid sequence of Fragment 3 and synthesized on an automated DNA synthesizer as described above.

Probe #1: A C N A C C A T [A/G] T C [T/C] T G [A/G] A T Probe #2: C A [A/G] G A [T/C] A T G G T N G T N G A

These probes are radioactively labeled and employed to screen the bovine genomic library constructed as follows: Bovine liver DNA is partially digested with the restriction endonuclease enzyme Sau 3A and sedimented through a sucrose gradient. Size fractionated DNA in the range of 15-30kb is then ligated to the lambda J1 BamH1 arms vector [Frischauf et al, <u>J. Mol. Biol.</u>, 170:827-842 (1983) Mullins et al., Nature 308: 856-858 (1984)]. The library is plated at 8000 recombinants per plate. Duplicate nitrocellulose replicas of the plaques are made and amplified according to a modification of the procedure of Woo et al, <u>Proc. Natl. Acad. Sci. USA</u>, 75:3688-91 (1978).

The radioactively labelled 17-mer Probe #1 is hybridized to the set of filters according to the following method:

The probe is kinased and hybridized to the other set of filters in 3M tetramethylammonium chloride (TMAC), 0.1M sodium phosphate pH6.5, 1mM EDTA, 5X Denhardts, 0.6% SDS, 100ug/ml salmon sperm DNA at 48 degrees C, and washed in 3M TMAC, 50mM Tris pH8.0 at 50 degrees C. These conditions minimize the detection of mismatches to the probe pool [see, Wood et al, <u>Proc. Natl. Acad. Sci, U.S.A.</u>, 82:1585-1588 (1985)]. 400,000 recombinants are screened by this procedure. One duplicate positive is plaque purified and the DNA is isolated from a plate lysate of the recombinant bacteriophage designated lambda bP-21. Bacteriophage bP-21 was deposited with the ATCC under accession number ATCC 40310 on March 6, 1987. The bP-21 clone encodes the bovine growth factor designated bBMP-2.

The oligonucleotide hybridizing region of this bBMP-2 clone is localized to an approximately 1.2 kb Sac I restriction fragment which is subcloned into M13 and sequenced by standard techniques. The partial DNA sequence and derived amino acid sequence of this Sac I fragment and the contiguous Hind III-Sac I restriction fragment of bP-21 are shown below in Table II. The bBMP-2 peptide sequence from this clone is 129 amino acids in length and is encoded by the DNA sequence from nucleotide #1 through nucleotide #387. The amino acid sequence corresponding to the tryptic fragment isolated from the bovine bone 28 to 30kd material is underlined in Table II. The underlined portion of the sequence corresponds to tryptic Fragment 3 above from which the oligonucleotide probes for bBMP-2 are designed. The predicted amino acid sequence indicates that tryptic Fragment 3 is preceded by a basic residue (K) as expected considering the specificity of trypsin. The arginine residue encoded by the CGT triplet is presumed to be the carboxy-terminus of the protein based on the presence of a stop codon (TAG) adjacent to it.

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#### TABLE II

5	(1) GGC G			GGG G	15 AAA K	GGA G	CAC H	CCT P	CTC L	30 CAC H	AGA R	AGA R	GAA E	X AAG	45 CGG R
		GCA A		CAC H	60 AAA K	CAG Q	CGG R	AAA K	CGC R	75 CTC L	AAG K	TCC S	AGC S	TGT C	90 AAG K
10		CAC H	CCT P	TTA L	105 TAT Y	GTG V	GAC D	TTC F	AGT S	120 GAT D	GTG V	GGG G	TGG W	AAT N	135 GAC D
15		ATC I		GCA A	150 CCG P	CCG P	GGG G	TAT Y	CAT H	165 GCC A	TTT F	TAC	TGC C	CAT	180 GGG G
	GAG E		CCT P	TTT F	195 CCC P	CTG L	GCC A	GAT D	CAC	210 CTT L		TCC S	ACG T	AAT N	225 CAT H
20	GCC A	ATT I	CTC V	CAA Q	240 ACT T	CTG L	GTC V	AAC N	TCA S	255 GTT V	AAC	TCT S	aag K	ATT I	270 CCC P
 <b>25</b>		GCA A	TGC C	TGT	385 GTC V	CCA	ACA	GAG E	CTC	300 AGC S	GCC A	ATC I	TCC S	ATG M	315 CTG L
		CTT L	GAT D	GAG E	330 AAT N	GAG	aag K	GTG V	GTA V	345 TTA L	AAG	AAC <u>N</u>		CAG O	360 GAC D
30	ATG M					TGT	GGG	TGT C	CGT	9) TAG	: CACA	397 GCA /	AAAT	4 'AAAA	07 TA
	TAA		417 ATA :	TATA:	4: 'ATA	27 TA T	TAGA	43 AAAA	7 C AG	CAAA	447 AAAA	TCA	AGTT	457 GAC	•
35	ACT	TTAA'	467 TAT '	TTCC	4 TAAT	77 GA A	GACT	48 TTAT	7 T TA!	rgga.	497 ATGG	AAT	GGAG.	AAA	
	AAG		517 ACA (	CAGC	5 TATT	27 IT G.	AAAA	53° CTAT	7 A TT	TATA'	547 ICTA		AAAA		
40	GTT		567 AAA	CAAA:		77 TT A	ATCA	58' GAGA		TTA					

## **EXAMPLE V**

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## **Human Bone Inductive Factors**

## A. hBMP-2: Class I and II

The HindIII-SacI bovine genomic bBMP-2 fragment described in Example IV A. is subcloned into an M13 vector. A <sup>32</sup>p-labeled single-stranded DNA probe is made from a template preparation of this subclone. This probe is used to screen polyadenylated RNAs from various cell and tissue sources.

Polyadenylated RNAs from various cell and tissue sources are electrophoresed on formaldehyde-agarose gels and transferred to nitrocellulose by the method of Toole et al., <u>supra</u>. The probe is then hybridized to the nitrocellulose blot in 50% formamide, 5 X SSC, 0.1% SDS, 40 mM sodium phosphate pH6.5, 100 µg/ml denatured salmon sperm DNA, and 5 mM vanadyl ribonucleosides at 42° C overnight and washed at 65° C in 0.2 X SSC, 0.1% SDS. Following autoradiography, a hybridizing band corresponding to an mRNA species of approximately 3.8 kb is detected in the lane containing RNA from the human cell line U-2 OS. The HindIII-SacI fragment is labeled with <sup>32</sup>P by nick translation and

used to screen the nitrocellulose filter replicas of a U-2 OS cDNA library by hybridization in standard hybridization buffer at 65° overnight followed by washing in 1 X SSC, 0.1% SDS at 65°.

This library was constructed by synthesizing cDNA from U-2 OS polyadenylated RNA and cloning into lambda gt10 by established techniques (Toole et al., supra). Twelve duplicate positive clones are picked and replated for secondaries. Duplicate nitrocellulose replicas are made of the secondary plates and both sets hybridized to the bovine genomic probe as the primary screening was performed. One set of filters is then washed in 1 X SSC, 0.1% SDS; the other in 0.1 X SSC, 0.1% SDS at 65°.

Two classes of hBMP-2 cDNA clones are evident based on strong (4 recombinants) or weak (7 recombinants) hybridization signals under the more stringent washing conditions (0.1 X SSC, 0.1% SDS). All 11 recombinant bacteriophages are plaque purified, small scale DNA preparations made from plate lysates of each, and the inserts subcloned into pSP65 and into M13 for sequence analysis. Sequence analysis of the strongly hybridizing clones designated hBHP-2 Class I (also known as BMP-2) indicates that they have extensive sequence homology with the sequence given in Table II. These clones are therefore cDNA encoding the human equivalent of the protein encoded by the bBMP-2 gene whose partial sequence is given in Table II. Sequence analysis of the weakly hybridizing recombinants designated hBMP-2 Class II (also known as BMP-4) indicates that they are also quite homologous with the sequence given in Table II at the 3' end of their coding regions, but less so in the more 5' regions. Thus they encode a human protein of similar, though not identical, structure to that above.

Full length hBMP-2 Class I cDNA clones are obtained in the following manner. The 1.5 kb insert of one of the Class Il subclones (II-10-1) is isolated and radioactively labeled by nick-translation. One set of the nitrocellulose replicas of the U-2 OS cDNA library screened above (50 filters, corresponding to 1,000,000 recombinant bacteriophage) is rehybridized with this probe under stringent conditions (hybridization at 65° in standard hybridization buffer; washing at 65° in 0.2 X SSC, 0.1% SDS). All recombinants which hybridize to the bovine genomic probe which do not hybridize to the Class II probe are picked and plaque purified (10 recombinants). Plate stocks are made and small scale bacteriophage DNA preparations made. After subcloning into M13, sequence analysis indicates that 4 of these represent clones which overlap the original Class I clone. One of these, lambda U2OS-39, contains an approximately 1.5 kb insert and was deposited with the ATCC on June 16, 1987 under accession number 40345. The partial DNA sequence (compiled from lambda U2OS-39 and several other hBMP-2 Class I cDNA recombinants) and derived amino acid sequence are shown below in Table III. Lambda U2OS-39 is expected to contain all of the nucleotide sequence necessary to encode the entire human counterpart of the protein BMP-2 Class I encoded by the bovine gene segment whose partial sequence is presented in Table II. This human cDNA hBMP-2 Class I contains an open reading frame of 1188 bp, encoding a protein of 396 amino acids. This protein of 396 amino acids has a molecular weight of 45kd based on this amino acid sequence. It is contemplated that this sequence represents the primary translation product. The protein is preceded by a 5' untranslated region of 342 bp with stop codons in all frames. The 13 bp region preceding this 5' untranslated region represents a linker used in the cDNA cloning procedure.

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# TABLE III

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	10 GTOGACTOTA		30 GCACITGG CIGGGGA	40 50 CIT CITGAACTIG	60 70 CAGGGAGAAT AACTTGGGCA
10	80 CCCCACTITG		100 ICCCCAC CCCACCC	110 120 TGC TTOSCCATCT	130 140 COGAGCOCCA COGCOCCICC
15	150 ACTOCTOGGC		170 IGAGACGC IGIICCC	180 190 AGC GIGAAAAGAG	200 210 AGACTGOGOG GOOGGGACOC
	220 GGGAGAAGGA	230 GGAGGCAAAG AAI	240 AAGGAACG GACATTO	250 260 GGT CCTTGCGCCA	270 280 GGTCCTTTGA CCAGAGTFTT
20	29.0 TCCATGTGGA	300 CGCICITICA AIC		320 330 IGC TICTTAGACG	340 350 GACTGCGGTC TCCTAAAGGT
25	(1) CGACC ATG ( MET (	370 FIG GCC GGG ACC Val Ala Gly Thr	) C CCC TGT CTT CT ATG CYS Leu Le	385 A GOG TTG CTG C U Ala Leu Leu L	400 TT CCC CAG GTC eu Pro Gln Val
30	CTC CTG GGG	415 C GGC GGG GCT G Gly Ala Ala G	430 GC CTC GTT CCG Sly Leu Val Pro	445 GAG CTG GGC CGC Glu Leu Gly Arg	AGG AAG TIC GCG Arg Lys Phe Ala
35	460 GCG GCG TCG Ala Ala Ser	475 TCC CGC CGC C Ser Gly Arg F	CC TCA TCC CAG	490 CCC TCT GAC GAG Pro Ser Asp Glu	505 GTC CTG AGC GAG Val Leu Ser Glu
	520 TTC GAG TTC Phe Glu Leu	coc cic cic a	535 GC ATG TTC GGC er MET Phe Gly	550 CTG AAA CAG AGA Leu Lys Gln Arg	565 CCC ACC CCC AGC Pro Thr Pro Ser
40	AGG GAC GCC	580 CGTG GTG CCC C Val Val Pro P	595 CC TAC ATG CTA Pro Tyr MET Leu 2	EAC CIG TAT CGC Asp Leu Tyr Arg	610 AGG CAC TOG GGT Arg His Ser Gly
45	625 CAG CCG GGO Gln Pro Gly	TOA COC GOC C	40 CA GAC CAC CGG ! TO ASP His Arg :	655 MTG GAG AGG GCA Leu Glu Arg Ala	670 GCC AGC CGA GCC Ala Ser Arg Ala
50	AAC ACT GTG Asn Thr Val	685 CCC AGC TTC C Arg Ser Phe H	700 AC CAT GAA GAA ' is His Glu Glu :	715 CCT TIG GAA GAA Ser Leu Glu Glu	CTA CCA GAA ACG Leu Pro Glu Thr

	730 AGT	GGG	AAA	AC	A AC	745	AGA	TTC	TTC	TT	760 AA1	TTP	AG1	TCI	' ATC	775 : œ	: ACC	GAG
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	CGG	TGG	ACT	GCA	CAG	GGA	CAC	GCC	AAC	CAT	GGA	TTC	GIG	GIG	GAA	GTG	GCC	CAC
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	CAC	<b>CA</b> A	СУП		1120		m~c	m~s	con c	1135	1.00				1150			
30	His	Gln	Asp	Glu	His	Ser	Trp	Ser	Gln	Ile	Aru	Pro	TIG	Leu	GIA Val	ACT	TTT Phe	GGC Gly
		1165																011
			GGA	AAA	GGG		1180 CCT	crc	CAC	AAA	AGA	1195 GAA	ΔΔΔ	رتات	CAA	CCC	1210 מממ	CAC
	His	Asp	Gly	Lys	Gly	His	Pro	Leu	His	Lys	Arg	Glu	Lys	Arg	Gln	Ala	Lys	His
35			]	1225				7	240					1255				
	AAA	CAG	$\alpha$	AAA	$\alpha$ c	CIT	AAG	TCC	AGC	TGT	AAG	AGA	CAC	CCT	TTG	TAC	GTG	GAC
	Lys	Gln	Arg	Lys	Arg	Leu	Lys	Ser	Ser	Cys	Lys	Arg	His	Pro	Leu	Tyr	Val	Asp
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1435 1450 1465 1480 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu

1495 1510 1525 AAT GAA AAG GIT GIA TTA AAG AAC TAT CAG GAC ATG GIT GIG GAG GGT TGT GGG Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly

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Full-length hBMP-2Class II human cDNA clones are obtained in the following manner. The 200 bp EcoRI-SacI fragment from the 5' end of the Class II recombinant II-10-1 is isolated from its plasmid subclone, labeled by nick-translation, and hybridized to a set of duplicate nitrocellulose replicas of the U-2 OS cDNA library (25 filters/set; representing 500,000 recombinants). Hybridization and washing are performed under stringent conditions as described

above. 16 duplicate positives are picked and replated for secondaries. Nitrocellulose filter replicas of the secondary plates are made and hybridized to an oligonucleotide which was synthesized to correspond to the sequence of II-10-1 and is of the following sequence:

CGGGCGCTCAGGATACTCAAGACCAGTGCTG

Hybridization is in standard hybridization buffer at 50°C with washing at 50° in 1 X SSC, 0.1% SDS. 14 recombinant bacteriophages which hybridize to this oligonucleotide are plaque purified. Plate stocks are made and small scale bacteriophage DNA preparations made. After subcloning 3 of these into M13, sequence analysis indicates that they represent clones which overlap the original Class II clone. One of these, lambda U2OS-3, was deposited with the ATCC under accession number 40342 on June 16, 1987. U2OS-3 contains an insert of approximately 1.8 kb. The partial DNA sequence and derived amino acid sequence of U2OS-3 are shown below in Table IV. This clone is expected to contain all of the nucleotide sequence necessary to encode the entire human BMP-2 Class II protein. This cDNA contains an open reading frame of 1224 bp, encoding a protein of 408 amino acids, preceded by a 5' untranslated region of 394 bp with stop codons in all frames, and contains a 3' untranslated region of 308 bp following the in-frame stop codon. The 8 bp region preceding the 5' untranslated region represents a linker used in the cDNA cloning procedure. This protein of 408 amino acids has molecular weight of 47kd and is contemplated to represent the primary translation

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product.

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# TABLE IV

5	10 CTCTAGAGGG					60 70 COGGAAGCIA GGIGAGIGIG
10	80 GCATCCGAGC		100 GAGOCTGAGA		120 GCTCCGGCTG	130 140 AGIATCIAGO TIGICIOCOC
	150 GATGGGATTC		170 TATCICGAGC		190 ACAGTOCCOG	200 210 GCCCTCGCCC AGGTTCACTG
15	220 CAACCGITCA	230 GAGGTCCCCA	240 GGAGCTGCTG	250 CIGGOGAGOC	260 CGCTACTGCA	270 280 GGGACCTATG GAGCCATTCC
20	290 GIAGIGCCAT	300 CCCCAGCAAC	310 GCACTGCTGC	320 AGCTTCCCTG	330 AGCCITTCCA	340 350 GCAAGITTGT TCAAGATTGG
25	360 CIGICAAGAA	370 TCATGGACIG	Ö8E ƏTATKITATT	390 CCTTCTTTTC	400 TCTCAACACA	(1) CC ATG ATT CCT MET Ile Pro
30	417 GGT AAC CC Gly Asn Arg	A ATG CTG AT J MET Leu ME	432 NG GIC GIT T NT Val Val I	TIA TIA TGC eu Leu Cys	447 CAA GIC CIG Gln Val Leu	462 G CTA GGA GGC GCG I Leu Gly Gly Ala
••	AGC CAT GC Ser His Ala	477 FAGT TTG AT A Ser Leu II	A CCT GAG	192 ACG GGG AAG Thr Gly Lys	507 AAA AAA GIC Lys Lys Val	C GCC GAG ATT CAG . Ala Glu Ile Gln
35	522 GGC CAC GCC Gly His Ala	53 GGA GGA CG A Gly Gly Ax	C OGC TCA C	552 GG CAG AGC Gly Gln Ser	CAT GAG CTC His Glu Leu	567 CCTG CGG GAC TTC Leu Arg Asp Phe
40	582 GAG GOG ACF Glu Ala Thr	CIT CIG CA	597 G ATG TIT G n MET Phe G	GG CTG CGC Cly Leu Arg	612 CSC CSC CCS Arg Arg Pro	627 CAG CCT AGC AAG Gln Pro Ser Lys
45	AGT GCC GTC Ser Ala Val	642 ATT COG GA Ile Pro As	C TAC ATG C p Tyr MET A	657 CCG GAT CTT LTG ASP Leu	TAC CGG CIT Tyr Arg Leu	672 CAG TCT GGG GAG Gln Ser Gly Glu
50	687 GAG GAG GAA Glu Glu Glu	GAG CAG AT Glu Gln Il	702 C CAC AGC A e His Ser T	CT GGT CTT	717 GAG TAT CCT Glu Tyr Pro	732 GAG CGC CGG GCC Glu Arg Pro Ala

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				747					762					777					
	AGC	ŒG	GCC	AAC	ACC	GTG	AGG	AGC			CAC	CAA	CAA			GAG	ממ	ATC	
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	Thr	Arg	Leu	Val	His	His	Asn	Val	Thr	Arq	Tro	Glu	Thr	Phe	Asp	Val	Ser	Pro	
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	722	100	TIA	<u>т.</u>	CAA	GGG	AGT	GGG	TAA	TGG	GCC	CAG	CIC	$\alpha$	$\infty$	CIC	CIG	GIC	
35	ALG	Ser	TEU	PIO	GII	GTÅ	Ser	Gly	Asn	Trp	Ala	Gln	Leu	Arg	Pro	Leu	Leu	Val	
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	Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser	
5	The same and the same same same same same same same sam	
5	1497 1512 1527 1542	
	GTC AAT TOO AGT ATC COO AAA GOO TGT TGT GTG COO ACT GAA CTG AGT GOO ATC	
	Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile	
10	1557 1572 1587	
	TOO ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG	
	Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu	
	1602 1617 (408) 1636 1646 1656	
	707, (400) 7030 7040 7030	
15	ATG GIA GIA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCITGA GGATAGACAG MET Val Val Glu Gly Cys Gly Cys Arg	
	and that the Gra Gra Gra Cys Arg	
	1666 1676 1686 1696 1706 1716 1726	=
	ATATACACAC CACACACACA CACCACATAC ACCACACA CACGITOCCA TOCACTCACC CACACACTAC	•
		•
20		
	1736 1746 1756 1766 1776 1786 1796	5
	ACAGACIGCT TOCTTATAGC TOGACTITTA TITAAAAAA AAAAAAAAA AATGGAAAAA ATOOCTAAAG	
	The first the second with million of a selection of the property of the second will be been a second beginning	٠.
25	1806 1816 1826 1836 1846 1856 1866 ATTCACCITG ACCITATITA TGACCITTACG TGCAAATGIT TTGACCATAT TGATCATATA TITTGACAAA	,
	" " " " " TO ACCITATION TO A THE ACTION TO A TOTAL TO A TOTAL AT A THING ACAM	١.
	1876 1886 1896 1906 1916 1926 1936	5
30	ATATATTTAT AACIACGIAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAA AAAAAAAAC	, P
	· · · · · · · · · · · · · · · · · · ·	•
	(	
	1946	
	CTAGAGTOGA OGGAATTC	

The sequences of BMP-2 Class I and II as shown in Tables II, III IV and have significant homology to the beta (B) and beta (A) subunits of the inhibins. The inhibins are a family of hormones which are presently being investigated for use in contraception. See, A. J. Mason et al, Nature, 318:659-663 (1985). To a lesser extent they are also homologous to Mullerian inhibiting substance (MIS), a testicular glycoprotein that causes regression of the Mullerian duct during development of the male embryo and transforming growth factor-beta (TGF-b) which can inhibit or stimulate growth of cells or cause them to differentiate. Furthermore, the sequence of Table IV encoding hBMP-2 Class II has significant homology to the Drosophila decapentaplegic (DPP-C) locus transcript. See, J. Massague, Cell, 49:437-438 (1987); R. W. Padgett et al, Nature, 325:81-84 (1987); R.L. Cate et al, Cell 45: 685-698 (1986). It is considered possible therefore that BMP-2 Class II is the human homolog of the protein made from this transcript form this developmental mutant locus.

#### **EXAMPLE VI**

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## Expression of Bone Inductive Factors.

In order to produce bovine, human or other mammalian bone inductive factors, the DNA encoding it is transferred into an appropriate expression vector and introduced into mammalian cells by conventional genetic engineering techniques.

One skilled in the art can construct mammalian expression vectors by employing the sequence of Tables II, III AND IV or other modified sequences and known vectors, such as pCD [Okayama et al., Mol. Cell Biol., 2:161-170 (1982)] and pJL3, pJL4 [Gough et al., EMBO J., 4:645-653 (1985)]. The transformation of these vectors into appropriate host cells can result in expression of osteoinductive factors. One skilled in the art could manipulate the sequences of Tables II, III and IV by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with bacterial sequences to create bacterial vectors for intracellular or extracellular expression by bacterial cells. For example, the coding sequences could be further manipulated (e.g. ligated to other known linkers or modified by deleting non-coding sequences there-from or altering nucleotides therein by other known techniques). The modified bone inductive factor coding sequence could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al., <u>Proc. Natl Acad. Sci. USA</u>, 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and bone inductive factor expressed thereby. For a strategy for producing extracellular expression of bone inductive factor in bacterial cells., see, e.g. European patent application EPA 177,343.

Similar manipulations can be performed for the construction of an insect vector [see, e.g. procedures described in published European patent application 155,476] for expression in insect cells. A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of the factors of the present invention by yeast cells. [See, e.g., procedures described in published PCT application W086/00639 and European patent application EPA 123,289].

A method for producing high levels of an osteoinductive factor of the invention from mammalian cells involves the construction of cells containing multiple copies of the heterologous bone inductive factor gene. The heterologous gene can be linked to an amplifiable marker, e.g. the dihydrofolate reductase (DHFR) gene for which cells containing increased gene copies can be selected for propagation in increasing concentrations of methotrexate (MTX) according to the procedures of Kaufman and Sharp, <u>J. Mol. Biol.</u>, 159:601-629 (1982). This approach can be employed with a number of different cell types.

For example, a plasmid containing a DNA sequence for a bone inductive factor of the invention in operative association with other plasmid sequences enabling expression thereof and the DHFR expression plasmid pAdA26SV(A) 3 [Kaufman and Sharp, Mol. Cell. Biol., 2:1304 (1982)] can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by calcium phosphate coprecipitation and transfection. DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum, and subsequently selected for amplification by growth in increasing concentrations of MTX (sequential steps in 0.02, 0.2, 1.0 and 5uM MTX) as described in Kaufman et al., Mol Cell Biol., 5: 1750 (1983). Transformants are cloned, and biologically active bone inductive factor expression is monitored by rat bone formation assay. Bone inductive factor expression should increase with increasing levels of MTX resistance. Similar procedures can be followed to produce other bone inductive factors.

Alternatively, the human gene is expressed directly, as described above. Active bone inductive factor may be produced in bacteria or yeast cells. However the presently preferred expression system for biologically active recombinant human bone inductive factor is stably transformed CHO cells.

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As one specific example, to produce the human bone inductive factor (hBMP-1) of Example V, the insert of U2OS-1 is released from the vector arms by digestion with Sal I and subcloned into the mammalian expression vector pMT2CX digested with Xho I. Plasmid DNA from this subclone is transfected into COS cells by the DEAE-dextran procedure [Sompayrac and Danna PNAS 78:7575-7578 (1981); Luthman and Magnusson, Nucl. Acids Res. 11: 1295-1308 (1983)]. Serum-free 24 hr. conditioned medium is collected from the cells starting 40-70 hr. post-transfection.

The mammalian expression vector pMT2 Cla-Xho (pMT2 CX) is a derivative of p91023 (b) (Wong et al., Science 228:810-815, 1985) differing from the latter in that it contains the ampicillin resistance gene in place of the tetracycline resistance gene and further contains a Xhol site for insertion of cDNA clones. The functional elements of pMT2 Cla-Xho have been described (Kaufman, R.J., 1985, Proc. Natl. Acad. Sci. USA 82:689-693) and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5' splice site and the majority of the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in E. coli.

Plasmid pMT2 Cla-Xho is obtained by EcoRl digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122. EcoRl digestion excises the cDNA insert present in pMT2-VWF, yielding pMT2 in linear form which can be ligated and used to transform <u>E</u>. coli HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods. pMT2CX is then constructed by digesting pMT2 with Eco RV and Xbal, treating the digested DNA with Klenow fragment of DNA polymerase I, and ligating Cla linkers (NEBiolabs, CATCGATG). This removes bases 2266 to 2421 starting from the Hind III site near the SV40 origin of replication and enhancer sequences of pMT2. Plasmid DNA is then digested with EcoRl, blunted as above, and ligated to an EcoRl adapter,

## 5' PO4-AATTCCTCGAGAGCT 3'

## 3' GGAGCTCTCGA 5'

digested with Xhol, and ligated, yielding pMT2 Cla-Xho, which may then be used to transform <u>E. coli</u> to ampicillin resistance. Plasmid pMT2 Cla-Xho DNA may be prepared by conventional methods.

#### Example VII

## Biological Activity of Expressed Bone Inductive Factor

#### 5 A. BMP-1

To measure the biological activity of the expressed bone inductive factor. (hBMP-1) obtained in Example VI above. The factor is partially purified on a Heparin Sepharose column. 4 ml of transfection supernatant from one 100 mm dish is concentrated approximately 10 fold by ultrafiltration on a YM 10 membrane and then dialyzed against 20mM Tris, 0.15 M NaCl, pH 7.4 (starting buffer). This material is then applied to a 1.1 ml Heparin Sepharose column in starting buffer. Unbound proteins are removed by an 8 ml wash of starting buffer, and bound proteins, including BMP-1, are desorbed by a 3-4 ml wash of 20 mM Tris, 2.0 M NaCl, pH 7.4.

The proteins bound by the Heparin column are concentrated approximately 10-fold on a Centricon 10 and the salt reduced by diafiltration with 0.1% trifluoroacetic acid. The appropriate amount of this solution is mixed with 20 mg of rat matrix and then assayed for <u>in vivo</u> bone and cartilage formation as previously described in Example III. A mock transfection supernatant fractionation is used as a control.

The implants containing rat matrix to which specific amounts of human BMP-1 have been added are removed from rats after seven days and processed for histological evaluation. Representative sections from each implant are stained for the presence of new bone mineral with von Kossa and acid fuschin, and for the presence of cartilage-specific matrix formation using toluidine blue. The types of cells present within the section, as well as the extent to which these cells display phenotype are evaluated.

Addition of human BMP-1 to the matrix material resulted in formation of cartilage-like nodules at 7 days post implantation. The chondroblast-type cells were recognizable by shape and expression of metachromatic matrix. The amount of activity observed for human BMP-1 was dependent upon the amount of human BMP-1 protein added to the matrix. Table IX illustrates the dose-response relationship of human BMP-1 protein to the amount of bone induction observed.

Table IX

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IMPLANT NUMBER	AMOUNT USED (equivalent of ml transfection media)	HISTOLOGICAL SCORE
876-134-1	10 BMP-1	C+2
876-134-2	3 BMP-1	C+1
876-134-3	1 BMP-1	C+/-
876-134-4	10 MOCK	C-
876-134-5	3 MOCK	C-
876-134-6	1 MOCK	C -

Cartilage (c) activity was scored on a scale from 0(-) to 5.

Similar levels of activity are seen in the Heparin Sepharose fractionated COS cell extracts. Partial purification is accomplished in a similar manner as described above except that 6 M urea is included in all the buffers. Further, in a

rat bone formation assay as described above, BMP-2 has similarly demonstrated chondrogenic activity.

The procedures described above may be employed to isolate other bone inductive factors of interest by utilizing the bovine bone inductive factors and/or human bone inductive factors as a probe source. Such other bone inductive factors may find similar utility in, inter alia, fracture repair.

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are believed to be encompassed within the claims appended hereto.

50 Claims

Claims for the following Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A gene encoding human BMP-2 comprising the following DNA sequence:

	10 GTOGACTOTA	20 GAGIGIGIGI -	30 CAGCACITGG	40 CTGGGGACTT	50 CTTGAACTTG	60 CAGGGAGAAT	70 AACITIGOGCA
5	80 CCCCACTITG	90 SCOOR	100 TTTGCCCAG	110 OGGAGOCIGC	120 TTCGCCATCT	130 COGAGOCOCA	140 coscocice
10	150 ACTOCTOGGC	160 CITGCCCGAC	170 ACTGAGACSC	180 TGITCCCAGC		200 AGACTGCGCG	210 GCCCGCACCC
15	220 GGGAGAAGGA	230 GGAGGCAAAG	240 AAAAGGAACG	250 GACATTCGGT	260 CCTTGCGCCA	270 GCICCITICA	280 CCAGAGITIT
20	(1) CGACC ATG G	OSCICITICA  IG GOO GGG	ATGGAOGIGI 370 ACC OGC TGI	CCCCCCTTCC 38	TTCTTAGACG 55 55 TTG CTG C	GACTGOGGTC ( 400 TT CCC CAG (	ne i de saes e e
25	MET VO	al Ala Gly 415 GGC GCG GC	Thr Arg Cys 4 I GGC CTC G	: Leu Leu Al 30 TT COG GAG	a Leu Leu I 445 CTG GGC CGC	eu Pro Gln '	Val
<b>30</b>	460 GCG GCG TCG Ala Ala Ser	TOG GGC GG Ser Gly Arg	T AOT OOO	490 CC CAG CCC : er Gln Pro :	TCT GAC GAG Ser Asp Glu	505 GIC CIG AGO Val Leu Ser	C GAG C Glu
35	520 TTC GAG TTG Phe Glu Leu	CGG CTG CTC Arg Leu Leu 580	535 CAGCATGT 1 Ser MET P	he Gly Leu :	550 AAA CAG AGA Lys Gln Arg	Pro Thr Pro	565 C AGC Ser
	AGG GAC GOC Arg Asp Ala	GTG GTG CCC	C CCC TAC AT Pro Tyr M	595 IG CTA GAC ( ET Leu Asp :	CTG TAT CGC Leu Tyr Arg	AGG CAC TOO Arg His Ser	GCT Gly
40	625 CAG CCG GGC Gln Pro Gly	TCA CCC GCC Ser Pro Ala	640 C CCA GAC CA A Pro Asp H	AC CGG TTG	655 EAG AGG GCA Glu Arg Ala	670 GCC AGC CGA Ala Ser Arg	· ccc
45	AAC ACT GIG Asn Thr Val	685 CGC AGC TTC Arg Ser Phe	CAC CAT GA	00 AA GAA TCT 1 Lu Glu Ser 1	715 FTG GAA GAA Leu Glu Glu	CTA CCA GAA Leu Pro Glu	ACG Thr

	730	)				745	5				760	)				775	5	
	AGI	' GGC	AA E	A AC	A ACC	: œ	AG	A TT	OTT	TT	LAA 1	TIP	AG	י דכי	אדע יו	$= \frac{1}{100}$	. AC	GAG
5	Ser	. Gl	/ Lys	3 Th	r Thr	Arc	Arg	g Phe	e Phe	Phe	e Asr	Lec	Sei	Se	r Ile	Pro	Thi	Glu
			790	)				809	5				820	)		-		835
	GAG	TTI	YEA '	) AC	CICA	GCA	GAC	CI	CAC	GII	TIC	CGA	GAZ	CAC	OTA E	CAZ	A GAT	COT
	Glu	Phe	: Ile	Thi	: Ser	Ala	Gli	ı Let	ı Glr	ı Val	. Phe	arg	, Gli	ı Gli	n MEI	Glr	1 Asp	Ala
10																	_	
	ATT	GGA	אאר	רבב י	850 מאלי		י יייי	י ב	1 ~ ~	865	) NOTE				880	) 		
	Leu	Gly	Ası	Ası	Ser	Ser	Phe	- UAL	: Hic	· Am	TIA	Yen:	TIC	. TAL	L GAA	VIA I	ATA	AAA Lys
		-								, ,,,,		- nau	1.1.6	- IYI	. GIU	TTE	: TTE	Lys
15		895					910					925	;				940	)
	CCT Down	GCA	ACA	$\cos \alpha$	AAC	TOG	AAA	TIC	$\infty$	GIG	ACC	AGI	CII	TIC	GAC	: ACC	: AGG	TTG
	PLU	ALA	unr	. YTS	ı Asn	ser	Tys	Phe	Pro	Val	Thr	Ser	Leu	Let	ı Asp	Thr	Arg	Leu
				955	i				970	1				985				
20	GIG	AAT	CAG			AGC	AGG	TGG	GAA	AGT	TTT	GAT	GIV	POS	, . ~~	. CC1	י כיווב	ATG
	Val	Asn	Gln	Asn	ı Ala	Ser	Arg	Tr	Glu	Ser	Phe	Asp	Val	Thr	Pro	Ala	Val	MET
	1000		λ. <u>.</u>			1015					1030					1045	i	
25	Ara	Tro	Thr	Ala	Gln	GGA	His	: GCC	AAC	CAT	GGA	TIC	GIG	GIG	GAA	GIG	GCC	CAC His
	170.01				1915		· .	- 17 /	. risii	. 1115	GIY	Pile	vai	vai	GIU	vaı	Ala	HIS
			1060					1075					ากคก			•		1105
	TIG	GAG	GAG	AAA	CAA	GGT	GIC	TOO	AAG	AGA	CAT	GIT	AGG	ATA	AGC	AGG	יווי אף	عست
	Leu	GIU	GIU	Lys	GIN	Gly	Val	Ser	Lys	Arg	His	Val	Arg	Ile	Ser	Arg	Ser	Leu
30					1120					1135					1150			
	CAC	CAA	GAT	GAA	CAC	AGC	TGG	TCA	CAG	ATA	AGG	CCA	באדו	בידים	1150 GTA	λCT	ىلىنىن	GGC
	His	Gln	Asp	Glu	His	Ser	Trp	Ser	Gln	Ile	Arg	Pro	Leu	Leu	Val	Thr	Phe	Gly
											_							<b></b> 1
35		.165 .CAT	CCA	מממ	ccc		1180		<i>~</i>			1195					1210	
	His	Asp	Glv	Lvs	Glv	His	Pm	Ten	Hie	AAA	ACA	GAA	AAA	œr	CAA	GCC	AAA	CAC His
		•	2	-1-	1				*****	цуз	мy	GIU	цуs	Arg	GIN	ALA	TÀZ	HIS
				1225				:	1240					L255				
40	AAA	CAG	œc	AAA	œc	CIT	AAG	TCC	AGC	TGT	AAG	AGA	CAC	CCT	TTG	TAC	GTG	GAC
	rys	GIN	Arg	TÀR	Arg	Leu	Lys	Ser	Ser	Cys	Lys	Arg	His	Pro	Leu	Tyr	Val	Asp
	1270				3	285				ר	300				,	226		
	TTC :	AGT	GAC	GIG			AAT	GAC	TGG	TTA	ere.	GCT	ccc	m	GGC	315 Tar	CAC	ccc
45	Phe :	Ser	Asp	Val	Gly	Trp	Asn	Asp	Trp	Ile	Val	Ala	Pro	Pro	Glv	Tyr	His	Ala
									_							-2-		
	، بنین		330	C2 C	CC3	~~~		345				1	360				נ	.375
	TTT ?	Ivr	Cvs	His	GGA	GAA Clu	TGC Ove	CCT Dm	TTT	Σ. ΩI.	CIG	GCT	GAT	CAT	CIG	AAC	TCC	ACT
50	Phe ?	-1-	J, 5	تسده	OLY (	JIU	-y5	FIO	FIIE	FIO	ıeu .	wrg .	wsb	uis	reu	ASN	ser	Inr
					390					405				1	420			
	AAT (	CAT	GCC .	ATT	GIT (	CAG .	ACG	TIG	GTC .	AAC '	TCT (	GIT :	AAC	TCT	AAG	TTA	CT.	AAG
	Asn I	us .	Ala	Ile	Val (	Gln '	Ihr	Leu	Val .	Asn :	Ser '	Val 1	Asn	Ser	Lys	Ile	Pro	Lys

1435 1450 1465 GCA TGC TGT GTC COG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu 5 1510 AAT GAA AAG GIT GIA TIA AAG AAC TAT CAG GAC AIG GIT GIG GAG GGT TGT GGG Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly 10 1573 1583 1593 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTITTAG AAAAAAGAAA Cys Arg AAAA 15 2. A gene encoding human BMP-2 having the amino acid sequence given in claim 1. 3. A gene encoding a protein exhibiting properties of human BMP-2 and comprising a DNA sequence: 20 (a) which differs from a DNA sequence of claim 1 in codon sequence due to the degeneracy of the genetic code; (b) which hybridises with a DNA sequence of claim 1 or section (a), above; or (c) represents a fragment, allelic or other variation of a DNA sequence of claim 1, whether said variation results in changes in the peptide sequence or not. 25 4. The DNA sequence of claim 3, which is a genomic DNA sequence. 5. The DNA sequence of claim 3, which is a cDNA sequence.

30 6. A gene encoding bovine BMP-2 comprising the following DNA sequence:

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5	(1) GGC G	CAC H	GAT	GGG G	15 AAA K	GGA G	CAC H	CCT P	CTC L	30 CAC H	AGA R	AGA R		AAG K	
5		GCA A		CAC H			CGG R			75 CTC L	AAG K	TCC S	AGC S	TGT C	90 AAG K
10	AGA R		CCT P	TTA L			GAC D			120 GAT D			TGG W	AAT N	135 GAC D
15	TGG W	ATC I	GTT V	GCA A	150 CCG P	CCG	GGG G	TAT Y	CAT H	165 GCC A	TTT F	TAC Y	TGC C	CAT H	180 GGG G
20	GAG E	С	CCT P	TTT	P	CTG L	GCC A	GAT D	CAC H	210 CTT L	AAC N	TCC S	ACG T	AAT N	225 CAT H
	A	Ι	V	CAA Q	240 ACT T	CTG L	V	N	S	V	AAC N	S	'AAG K		P CCC
<b>25</b>	AAG	GCA	TGC C	TGT	GTC	CCA	ACA T	GAG	CTC	AGC	GCC	ATC I	TCC	ATG M	315 CTG L
30	TAC Y	CTT L	GAT D	GAG. E	330 AAT N	GAG E	AAG K	GTG V	GTA V	345 TTA L	AAG K		TAT Y	CAG O	360 GAC D
35	ATG M	GTT V		GAG E	GGT	TGT	GGG G	TGT	(129 CGT R	) TAGO	CACAC	397 GCA A	\AATA	4 ( \AAA)	7 CA
	TAAA	TATA	17 TA 1	TATAT	42 ATAI	7 A TI	'AGA	437 \AAA(	ago	<b>LAAA</b> S	447 AAA	TCAA	4 AGTTO	57 SAC	
40 .	ACTI		67 AT 1	TCCC	47 AATG		ACTT	487 TATI	TAI	GGAA	497 TGG	AATG		507 AAA	
45	AAGA	AAAA	17 .CA C	AGCT	52 ATTT 57	T GA	AAAC			TATA	547 CTA	CCGA		557 SAA	
	GTTG			TAAA			TCAG		ATT A	TT					

50 7. A gene encoding bovine BMP-2 containing the amino acid sequence of claim 6.

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- 8. A gene encoding a protein exhibiting properties of bovine BMP-2 and comprising DNA sequences:
  - (a) which differ from a DNA sequence of claim 7 in codon sequence due to the degeneracy of the genetic code;
  - (b) which hybridise with a DNA sequence of claim 7 or section (a), above; or
  - (c) represent fragments, allelic or other variations of a DNA sequence of claim 7, whether said variations result in changes in the peptide sequence or not.

- 9. The DNA sequence of claim 8, which is a genomic DNA sequence.
- 10. The DNA sequence of claim 8, which is a cDNA sequence.

5 11. A gene encoding human BMP-4 comprising the following DNA sequence:

	10	20	30	40	50	. 60	70
	CTCTAGAGGG				GGAGCCCGGC	COGGAAGCTA	GGIGAGIGIG
10							
		00	100	330	120	120	140
					GCTCCGGCTG		
	GCATCUGAGC	TENGGAMUSC	GAGCCIGAGA	GGGGGGG	GCICUGGCIG	MINICIANC	116101000
15	150	160	170	180	190	200	210
	GATGGGATTC	COCTICCAAGC	TATCTOGAGE	CICCACCCC	ACAGTCCCCG	COCCIOCOC	AGGITCACIG
	220	230	240	250	260	270	280
20					OGCTACTGCA		
	anacia.	~~~~~.	<u></u>	<b>5.005</b>			
						242	252
					330		
	GIAGIGCCAT	CCCGAGCAAC	GCACIGCIGC	AGCITCOCIG	AGCCTTTCCA	GCAAGITIGI	TCAAGATIGG
25							
	360	370	380	390	400	(1)	aga karaja ara dan karabah.
	CIGICAAGAA	TCATGGACIG	TUATUATATG	CCITCITITIC	TGTCAAGACA	CC ATG ATT	CCT
					ها در مید شود بیودین در در	MET .Ile	Pro

		417					432					447					462	
	GGT	AAC	<b>CGA</b>	ATG	CIG	ATG	GIC	GIT	TTA	TTA	TGC	CAA	GTC	CTG	CTA	GGA	GGC	GCG
	Gly	Asn	Arg	MET	Leu	MET	Val	Val	Leu	Leu	Cys	Gln	Val	Leu	Leu	Gly	Gly	Ala
5				455												•		
	acc.	רשעה	CCTI	477	m	BATTS	~~		492					507				
	Sor	Hic	Ala GCI	SO.	Tan	TIA	001	فکھتا ' دداہ	ACG	GGG	AAG	AAA	AAA	GIC	GCC	GAG	ATT	CAG
	Ser	1113	ма	SeT	TEU	116	PIO	GIU	шп	GIĀ	тЛя	rys	TÀ2	vaı	ALA	GIU	TTE	Gln
	522					537					552					567		
10		CAC	GCG	GGA	GGA			TCA	GGG	CAG	332	СУТ	GAG	CIIC	CTC:	567 CCC	GAC	TTC
	Gly	His	Ala	Gly	Gly	Arq	Arg	Ser	Glv	Gln	Ser	His	Glu	Teu	Ten	Am	Asn	Phe
	_			_	_	_	_		•									
			582					597					612					627
15	GAG	GCC	ACA	CIT	CIG	CAG	ATG	$\mathbf{T}\mathbf{T}\mathbf{T}$	GGG	CIG	$\alpha$ C	$\alpha$ c	$\alpha$ c	$\infty$	CAG	$\alpha$ T	AGC	AAG
	GIU	Ala	Thr	Leu	Leu	Gln	MET	Phe	Gly	Leu	Arg	Arg	Arg	Pro	Gln	Pro	Ser	Lys
					<i>-</i> 40													
	ΔζΤ	ccc	CTC	יוינימ	642	CAC	TITA CT	אמעי	~~	657	~	m» a	~~~	~~~	672			~~
	Ser	Ala	Val	Tie	Pm	yen auc	TAC	MEG	722	QAT.	CIT	TAC	7200	CIT	CAG	TCT	Clu	GAG Glu
20	-		V C.		110	A L	<b>T</b> YL	PILI	My	vəh	Leu	ığı	Arg	IEU	GIII	Ser	GTĀ	GIU
		687					702					717					732	
	GAG	GAG	GAA	GAG	CAG	ATC		AGC	ACT	GGT	CIT		TAT	CCT	GAG	œς	œ;	GCC
	Glu	Glu	Glu	Glu	Gln	Ile	His	Ser	Thr	Gly	Leu	Glu	Tyr	Pro	Glu	Aru	Pro	Ala
													•					
25				747					762					777				
	AGC	œ	GCC			GTG	AGG	AGC			CAC	GAA	CAA	777	CTC:	CAC	אאר	ATC
	Ser	Arg	Ala	Asn	Thr	Val	Arg	Ser	Phe	His	His	Glu	Glu	His	Ten	Clu	yen wwc	Ile
•		_														~J1U	ASIL	TIE
00	792					807					822					837		
30	CCA	GGG	ACC	AGT	GAA	AAC	TCT	GCT	$\mathbf{T}\mathbf{T}\mathbf{T}$	ŒT	TTC	CTC	TTT	AAC	CTC	AGC	AGC	ATC
	Pro	GTĀ	Thr	Ser	Glu	Asn	Ser	Ala	Phe	Arg	Phe	Leu	Phe	Asn	Leu	Ser	Ser	Ile
			852					0.00										
	cer	GAG		GAG	CITC.	איני	<b>™</b> ~	867	cm	CAC	CTTTP	~~	882		~~~			897 GTG
35	Pro	Glu	Asn	Glu	Val	Tle	Sor	Sor	Ala	Clu	Ton	λ	Tou	The	3	GAG	CAG	Val
									ma	GIU	124	wid	LEU	FILE	Arg	GIU	GIN	val
					912					927					942			
	GAC	CAG	GGC	CCT	GAT	TGG	GAA	AGG	GGC	TIC	CAC	CT	ATA	AAC	ATT	TAT	GAG	GIT
	Asp	Gln	Gly	Pro	Asp	Trp	Glu	Arg	Gly	Phe	His	Arg	Ile	Asn	Ile	Tyr	Glu	Val
40																-		
	NIDC.	957	~~	~~		-	972					987				- :	1002	
	WELL	AAL	Dm	CCA D	GCA	GAA	GIG	GIG	CT	GGG	CAC	CIC	ATC	ACA	<b>Œ</b> A	CIA	CTG	GAC
	MOT	тÃ2	PLO	PIO	Ala	GIU	vaı	vai	Pro	GTĀ	His	Leu	Ile	Thr	Arg	Leu	Leu	Asp
			1	1017				,	1032				,	0.47				
45	ACG	AGA			CAC	CAC	<b>አል</b> ጥ	ന്ദ്ര	ACA	ന്ദ	Trace.	GAA	س لا	.047	CAM	cur-	300	~~
	Thr	Arg	Leu	Val	His	His	Asn	.Val	Thr	Ara	Tro	Glu	Thr.	Dhe	yez.	01G	Sor	Dm.
•				٠			7 6.7	: Tage	\ <del>\```</del>			<u></u> ,	, 44,44,	,,,,,,,	برچہ	vai.	عجر.	P10
	1062					.077				3	092				1	107		
	GOG	GIC	CIT	$\alpha$ C	ŢĢĢ	ACC	$\alpha$	GAG	AAG .	CAG	CCA	AAC	TAT	GGG	CTA.	GCC	ATT.	GAG
50	Ala	Val	Leu	Arg	Trp	Thr	Arg	Glu	Lys	Gln	Pro	Asn	Tyr	Gly	Leu .	Ala	Ile	Glu
								_						_				
	יינב		.122	~~	<b>~~</b>	<b>~</b> ~		.137		<b>~</b> ~	_a ÷	1	152				1	167
	GIG . Val	ひして	LAU His	CIU	CAT.	CAG	ACT	CGG	ACC mb	CAC	CAG	GGC	CAG	CAT	GIC .	AGG	TTA	AGC
E E	Val		جىد،	لمصد	ицы	GIL	ПΙ	wid	mr	การ	GII	GIŻ	GIN	His	Val .	Arg	Ile	Ser

	1182 1197 1212
5	OGA TOG TTA CCT CAA GOG AGT GOG AAT TOG GOC CAG CTC CGG CCC CTC CTG GTC Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
	1227
	ACC TIT GGC CAT GAT GGC CGG GGC CAT GCC TIG ACC CGA CGC CGG AGG GCC AAG
	Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys
10	2007
	OGT AGC COT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG
	Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
	1332
15	CGC CAC TOG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TCG ATT GTG
	Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
	1392 1407 1422 1437
	GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTTT CCA CTG
20	Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu
	1452 1467 1482
	GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser
25	1407
	1497 1512 1527 1542 GTC AAT TOO AGT ATC COO AAA GOO TGT TGT GTG COO ACT GAA CTG AGT GOO ATC
	Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
	1557 1572 1587
30	TOO ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
	Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
	1602 1617 (408) 1636 1646 1656
	ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
35	MET Val Val Glu Gly Cys Gly Cys Arg
	1666 1676 1686 1696 1706 1716 1726
	ATATACACAC CACACACACA CACCACATAC ACCACACA CACGITOCCA TOCACTOACC CACACACTAC
40	1736 1746 1756 1766 1776 1786 1796
	1736 1746 1756 1766 1776 1786 1796 ACAGACTGCT TOCTTATAGC TOGACTTTTA TITTAAAAAAA AAAAAAAAA AATGGAAAAA ATCCTAAAC
:	atental see an makanna majiji sumaa sa militika digumahka a kulomiganin an assa dike mina maa a sa simula iyam
	1806 1816 1826 1836 1846 1856 1866
45	ATTCACCTIC ACCITATITA TGACTITACG TGCAAATGIT TTGACCATAT TGATCATATA TTTTGACAAA
	1876 1886 1896 1906 1916 1926 1936
50	ATATATTAT AACTACGIAT TAAAAGAAAA AAATAAAATG AGTCATTATT TIAAAAAAAA AAAAAAAACT
-	1946
	CTAGAGYCGA CGGAATYC
55	
12	2. A gene encoding human BMP-4 having the amino acid sequence given in claim 11.

13. A gene encoding a protein exhibiting properties of BMP-4 and comprising a DNA sequence:

- (a) which differs from a DNA sequence of claim 11 in codon sequence due to the degeneracy of the genetic code:
- (b) which hybridises with a DNA sequence of claim 11 or section (a), above; or
- (c) represents a fragment, allelic or other variation of a DNA sequence of claim 11, whether said variation results in changes in the peptide sequence or not.
- 14. The DNA sequence of claim 13, which is a genomic DNA sequence.
- 15. The DNA sequence of claim 13, which is a cDNA sequence.
- 16. A vector containing the gene or DNA sequence of any one of claims 1 to 15 in operative association with an expression control sequence.
- 17. A cell transformed with a vector of claim 16.
- 18. The cell of claim 17 which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.
- 19. The cell of claim 18 which is a CHO cell.
- 20. A protein exhibiting properties of BMP-2 which is encoded by a gene or DNA sequence of any one of claims 1 to 10.
  - 21. A protein exhibiting properties of BMP-2, which is obtainable by the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence of any one of claims 1 to 10, and recovering said protein from said culture medium.
  - 22. A protein exhibiting properties of BMP-4 which is encoded by a gene or DNA sequence of any one of claims 11 to 15.
  - 23. A protein exhibiting properties of BMP-4, which is obtainable by the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence of any one of claims 11 to 15, and recovering said protein from said culture medium.
    - 24. A process for producing the protein of claims 21 or 23, comprising the steps of culturing in a suitable culture medium the cell of claim 17 and isolating said protein from said culture medium.
- 25. A pharmaceutical composition comprising the proteins of any one of claims 20 to 23, individually or in combination, and a pharmaceutically acceptable vehicle.
  - **26.** The pharmaceutical composition of claim 25, further comprising a matrix capable of delivering the composition to the site of the bone or cartilage defect and providing a structure for inducing bone or cartilage formation.
  - 27. The pharmaceutical composition of claim 26, wherein said matrix comprises hydroxyapatite, collagen, polylactic acid or tricalcium phosphate.
- **28.** Use of a protein of any one of claims 20 to 23, individually or in combination, for the preparation of a pharmaceutical composition for inducing bone or cartilage formation.

#### Claims for the following Contracting State: AT

A process for the preparation of a gene encoding human BMP-2 comprising the following DNA sequence:

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5	10 GTOGACTOTA	20 GAGTGTGTGT	30 CAGCACITGG	40 CTGGGGACIT	50 CTIGAACTIG	60 CAGGGAGAAT	70 AACTIGGGCA
	80 CCCACTITG	00 90	100 TITGCCCAG	110 CGGAGCCTGC	120 TTGGCCATCT	130 CCCACCCCCA	140
10	150 ACTOCTOGGC	160 CTTGCCCGAC	170 ACTGAGACGC	180 TGTTCCCAGC	190 GTGAAAAGAG	200 AGACTGOGOG	210 GCCCGCACCC
15	220 GGGAGAAGGA	230 GGAGGCAAAG	240 AAAAGGAAGG	250 CACATTOGGT	260 CCTTGCGCCA	270 GGTCCITICA	
20	290 TCCATGTGGA	300 CCCTCTTTCA	7-7	ccccccrcc	OEE COADATIOTT	340 GACTGOGGTC	350 TOCTAAAGGT
20	(1) CGACC ATG ( MET V	FTG GCC GGG Val Ala Gly	370 ACC CGC TGI Thr Arg Cys	38 CTT CTA GC Leu Leu Al	5 G TTG CTG C a Leu Leu I	400 TT CCC CAG eu Pro Gln	GTC Val
25	CTC CTG GGC Leu Leu Gly	415 GGC GGG GG Gly Ala Al	T GGC CTC G	30 TT COS GAG al Pro Glu	445 CTG GGC GGO Leu Gly Arg	AGG AAG TI	c ccc e Ala
30	460 GCG GCG TCG Ala Ala Ser	47 TOG GGC OG Ser Gly Ar	C CCC TCA I	490 CC CAG CCC er Gln Pro	TCT GAC GAG Ser Asp Glu	505 GTC CTG AG Val Leu Se	C GAG r Glu
35		cos crs cr			550 AAA CAG AGA Lys Gln Arg		
					CIG TAT CGC Leu Tyr Arg		
<b>40</b> .	625 CAG CCG GGC Gln Pro Gly			AC OGG TTG	655 GAG AGG GCA Glu Arg Ala		A GCC
45	AAC ACT GTG Asn Thr Val		C CAC CAT G			CITA CCA GA	

	730					745	5				760	)				775		
	AG.	C_GC	g aa	A AC	A AO	C 030	AG	A TT	e m	c m	ראג יו	مليل م	4 - AC	ייער יין	ኮ አጥ	~~	· 2~	GAG
5	Sei	- G1	у Іу	's Th	r Th	c Arc	Ar	g Phe	Pho	e Phe	e Ası	Le	ı Sei	r Se	r Ile	e Pro	Thr	. Glu
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	GAC	TI	T AT	C AC	CTO	A GCA	GAC	CIT	ר כאנ	GI	י דוכ	CGZ	GAZ	C3/	ידב :	CAA	CAT	835 GCT
	Glu	Ph	e Il	e Th	r Sei	: Ala	Gli	ı Leu	ı Glı	val	Phe	Arc	Glu	ı Glı	1 MET	Gln	Aso	Ala
10																		
	TTA	GG	A AA	ימג ר	850 מכל יד	י אכדו י	1	- C20		865					880	)		
	Leu	GI	y As	n Asi	n Sei	Ser	Phe	- CAI	CAU	- Cua	All	· AAU	ATI	' TAI	GAZ	ATC	ATA	AAA Lys
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15		89		_			910	)				925	;				940	
	CI C	GC	A AC	A GO	CAAC	TOG	AAA	TIC	$= \infty$	GIG	ACC	AGI	CII	TIC	GAC	ACC	300	m-
	PIO	AL	a uni	C AL	a Ast	Ser	Lys	Phe	Pro	Val	Thr	Ser	Leu	Let	ı Asp	Thr	Arg	Leu
				955	5				970					005				
20	GTG	AA	CAC	G AAT	r GCA	AGC	AGG	TGG	CAA	ACT	4444	GAT	CITY	985	• ~~	· com	CTIC:	ATG
	Val	Ası	ı Gli	a Ast	ı Ala	Ser	Arg	Trp	Glu	Ser	Phe	Asp	Val	Thr	Pro	Ala	Val	MET
	100																	
			 			1015				-	1030					1045		
05	Arq	Tr	Thi	Ala	Gln	GUA	Hie	Ala	AAC	CAT	GGA	TIC	GIG	GIG	GAA	GIG Val	GCC	CAC
25	•	•					حبيده	ALC.	VZII	. nis	GTÅ	Pile	var	val	GIU	val	Ala	His
			1060					1075					1090				-	1105
	TIG	GAC	GAC	AAA	CAA	GGT	GIC	TCC	AAG	AGA	CAT	GTT	AGG	ATA	AGC	AGG	<b>~</b> ™	m
	Leu	GIU	GIU	l Lys	GIn	Gly	Val	Ser	Lys	Arg	His	Val	"Arg	Ile	Ser	Arg	Ser	Ten
30					1120					1135					3356			
	CAC	CAA	GAI	' GAA	CAC	AGC	TGG	TCA	CAG	ATA	AGG	$\alpha$	באות	CTTS	1150 CTD	ACT	TTTTT	~~
	His	Gln	Asp	Glu	His	Ser	Trp	Ser	Gln	Ile	Arq	Pro	Leu	Leu	Val	Thr	Phe	GGC
											,				_			CLY
35		165 CAT		222	~~~		.180					195				. 1	210	
	His	Asp	Glv	Ive	Glv	Hie.	Dm.	CIC	CAC	AAA	AGA	GAA	AAA	CI	CAA	œ	AAA	CAC
			<b>4</b>	-2,0	OLY		FIG	TEU	nıs	TÃ2	Arg	GIU	TÀZ	Arg	GIN	Ala	Lys	His
				1225				1	240				1	255				
40	AAA	CAG	œc	AAA	œc	CIT .	AAG	TCC	AGC	TGT	AAG	AGA	CAC	CCT	TTG	TAC	GIG	GAC
	TÄZ	GIN	Arg	Lys	Arg	Leu	Lys	Ser	Ser	Cys	Lys .	Arg	His	Pro	Leu	Tyr '	Val .	Asp
	1270				1	285				,	200				_			
	TTC.	AGT	GAC	GTG	GGG	TGG	AAT	GAC	TCC	יייינע. ד	300 CTG /	دس	$\sim$	~~	1	.315 TAT (	~~~	~~~
45	Phe :	Ser	Asp	Val	Gly	Trp .	Asn.	Asp '	Trp	Ile	Val	Ala	Pro	Pro	Glv	Tyr i	LAC (	3CC 81a
					_	-		•	-							*J* .	، حيد	-La
	، بنیلی		.330	<b>~</b>	005	<b></b>		345				1	360				1:	375
	TTT ?	DY-	1GC	UAC Hic	Clus	GAA :	IGC	CT!	III -	CCT (	CIG (	GCT (	GAT (	CAT	CIG .	AAC :	rcc 1	ACT
50	Phe :	-1-	~ <u>y</u> 3	•112	OTA (	oru (	Jys .	PIO.	me	, סוצ	Leu i	·ua.	( dzA	HIS	Leu .	Asn S	Ser :	וחד
- <del>-</del>				1	390				1.	405				1	420			
	AAT (	ΆΤ	cc	TTA	GTT (	CAG I	ice :	rig (	FIC :	AAC :	rcr (	TT I	AAC 1	ICT .	AAG	ATT C	XT Z	VAG
	Asn I	lis .	Ala	Ile	Val (	Sln 1	hr 1	Ceu 1	/al /	Asn S	Ser 1	/al /	Asn S	Ser	Lys :	Ile I	ro I	.ys
																		-

1435 1450 1465 1480 GCA TGC TGT GTC CCG-ACA-GAA-CTC-AGT GCT ATC TCG ATG-CTG TAC CTT GAC GAG Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu 5 1510 1525 1495 ANT CAA AAG GIT GIA TIA AAG AAC TAT CAG GAC ATG GIT GIG GAG GGT TGT GGG Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly 10 1540 (396) 1553 1563 1573 1583 1593 TGT CCC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA Cys Arg

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wherein said process comprises the following steps:

- a) screening of a gene library constructed from U-2 OS derived DNA or cDNA with a labelled bBMP-2 fragment by hybridization,
- b) isolating positive clones, and
- c) isolating the DNA-inserts from said clones.
- 2. The process according to claim 1, wherein the gene encodes human BMP-2 having the amino acid sequence given in claim 1.
  - 3. A process for the preparation of a gene encoding a protein exhibiting properties of human BMP-2 and comprising a DNA sequence:
    - a) which differs from a DNA sequence of claim 1 in codon sequence due to the degeneracy of the genetic code;
    - b) which hybridizes with a DNA sequence of claim 1 or section (a), above; or
    - c) represents a fragment, allelic or other variation of a DNA sequence of claim 1, whether said variation results in changes in the peptide sequence or not,
- 35 wherein said process comprises standard techniques of molecular biology.
  - 4. The process according to claim 3, wherein the DNA sequence is a genomic DNA sequence.
  - 5. The process according to claim 3, wherein the DNA sequence is a cDNA sequence.
  - 6. A process for the preparation of a gene encoding bovine BMP-2 comprising the following DNA sequence:

5	(1) GGC G	CAC H	GAT D	GGG G	15 AAA K	GGA G	CAC H	CCT P	CTC L	30 CAC H	AGA R		GAA E	AAG K	45 CGG R
	CAA Q	GCA A	AAA K	CAC H	60 <b>AAA</b> K	CAG Q	CGG R	AAA K	CGC R	75 CTC L	AAG K	TCC S	AGC S	TGT C	90 AAG K
10	AGA R	CAC H	CCT P	TTA L	105 TAT Y	GTG V		TTC F		120 GAT D			TGG W	AAT N	135 GAC D
15	TGG W	ATC I	GTT V	GCA A	150 CCG P		GGG G			165 GCC A	TTT	TAC		CAT	
20	GAG E	С	CCT P	TTT	₽	CTG L	GCC A	GAT D	CAC	210 CTT L	AAC N	TCC S	ACG T	AAT N	225 CAT H
25	GCC.	ATT I	CTC V	CAA	240 ACT	CTG:	GTC V	AAC N	TCA S	255 GTT V	AAC		AAG K		270 CCC P
	AAG K	GCA A	TGC C	TGT C	385 GTC V	CCA P	ACA T	GAG E	CTC L	300 AGC S	GCC A	ATC I	TCC S	ATG M	315 CTG L
30	TAC Y	CTT L	GAT D		330 AAT N	GAG E	AAG K	GTG V	GTA V	345 TTA L	AAG K	AAC N	TAT Y		
35	ATG M	GTT V	GTC V	GAG E	GGT	TGT	GGG G	TGT	(129 CGT R	TAGO	3 ACAG	97 CA A	AATA	4 C LAAAT	)7 :A
40	TAAA	ATATA				'A TT	'AGAA	AAAC	AGC	AAAA	AAA	TCAA	GTTG		
•	ACTI			TCCC			ACTT			'GGAA		AATG		107 .A.A	
45	AAGA		17 .CA C	AGCT	52 ATTT		AAAC	537 TATA		'ATAT	547 CTA	CCGA		57 AA	
	GTTG	_	67 AA C	AAAT	57 ATTT		TCAG	587 AGAA		TT,					

wherein said process comprises the following steps:

a) screening a gene library constructed from bovine liver DNA or cDNA with a labelled probe designed on the basis of the amino acid sequence of a fragment of bBMP-2,

b) isolating positive clones, and

c) isolating the DNA-inserts from said clones.

<sup>7.</sup> The process according to claim 6, wherein the gene encodes bovine BMP-2 having the amino acid sequence of claim 6.

	8.	A process for the prepar DNA sequences:	ation of a gene	encoding a p	rotein exhibiting	properties of bov	ine BMP-2 and comprising
5	_	<ul><li>b) which hybridize w</li></ul>	rith a DNA sequ nts, allelic or oth	dence of claim ner variations	7 or section a),	above; or	neracy of the genetic code
10		wherein said process co	mprises standa	ard techniques	of molecular bi	ology.	
	9.	The process according t	o claim 8, whe	rein the DNA s	sequence is a g	enomic DNA sequ	Jence.
	10.	The process according t	o claim 8, whe	rein the DNA s	sequence is a cl	ONA sequence.	
15	11.	A process for the prepar	ation of a gene	encoding hur	man BMP-4 com	prising the follow	ring DNA sequence:
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	CICIA	1( GAGG		CYCC	20 AGGA	GGG	AGGG	30 AGG	GAAG		40 3C G	GAGC	50 3333		ĠŸ¥(	60 CTA	GGT	70 AGIGIG
5	GCATO	80 CGAGO	-	aggg.	90 ACCC	GAG		100 AGA	œœ		10 CT G	CICC	120 GCTG		PATCI	130 PAGC	TIG	140 CTCCCC
10	GATGG	150 GATTO		JCC.	160 AAGC	TAT		170 AGC	crca		30 X A	CAGIY	190		œrœ	200 \$	agg)	210 TCACIG
15	CAACC	220 3TTC2		3610	230 0007A	GGA		240 CIG	CIGG		50 CC O	GCIA	260 CIGCA		SACCI	270 'ATG	GAGO	280 CATTCC
	GIAGI	290 CCA1		CCAC	300 CAAC	GCA		310 IGC	AGCI		20 IG A	GCCIT	330 PTCCA		<b>V</b> AGITI	340 TGT		350 CATTGG
<b>20</b>	CICICI	360 AAGAZ		ATCC:	370 ACIG			380 ATG			90 TC T	GICA	400 AGACA	$\infty$			CCT Pro	
25	GT Gly	417 AAC Asn	yrd Ccy	atg Met	CIG Leu	atg Met	432 GTC Val	GIT	TTA Leu	TTA Leu	TGC Cys	447 CAA Gln	GTC Val	CIG Leu	CIA Leu	GGA GGA	462 GGC Gly	GCG Ala
30	AGC Ser	CAT His	GCT Ala	477 AGT Ser	TTG Leu	ATA Ile	CCT Pro	GAG Glu	492 ACG Thr	GGG Gly	aag Lys	aaa Lys	aaa Lys	507 GTC Val	GCC Ala	GAG Glu	ATT Ile	CAG Gln
35	522 GGC Gly	CAC	SOS Ala	GGA Gly	GGA Gly	537 CGC Arg	OGC Arg	TCA Ser	GGG Gly	CAG Gln	552 AGC Ser	CAT	GAG Glu	CTC Leu	CIG Leu	567 CGG Arg	GAC Asp	TTC Phe
	GAG Glu	GOG Ala	582 ACA Thr	CIT Leu	Leu CIG	CAG Gln	ATG MET	597 TTT Phe	GGG	CIG Leu	OGC Arg	OGC Arg	612 CGC Arg	ccc Pro	CAG Gln	CCT Pro	AGC Ser	627 AAG Lys
40	AGT Ser	G∝ Ala	GTC Val	ATT Ile	642	GAC Asp	TAC Tyr	ATG MET	ogg Arg	657 GAT ASP	CIT Leu	TAC Tyr	CGG Arg	CIT Leu	672 CAG Gln	TCT Ser	GGG Gly	GAG GAG
45	GAG Glu	687 GAG Glu	GAA Glu	GAG Glu	G]n	ATC Ile	702 CAC His	AGC Ser	ACT Thr	GGT Gly	CIT Leu	717 GAG Glu	TAT Tyr	CCT Pro	GAG Glu	OGC Arg	732	GCC Ala

				/4/					762					777				
			GΩ															
	Ser	Arg	Ala	Asn	Thr	Val	Arg	Ser	Phe	His.	His	Glu	Glu	His	-Leu	-Glu	Asn	He
5	702															007		
	792	~~~				807					822	~~~	~~~~		~~~	837	300	NOC.
			ACC															
	Pro	GTĀ	Thr	ser	GIU	ASD	Ser	ALA	Fne	Arg	me	Leu	rne	ASI	Leu	Ser	Ser	ше
			852					067	•				001					897
10	COLIFE COLIFIE	CNG	AAC	CNC	~	ארואר	m~	867	cm	CNC	com.	~~	882	m	~	CNG	CAG	
			Asn															
	FIU	Giu	ASII	Giu	Val	тe	Ser	Ser	ALIA	GIU	TELL	ALG	TEIL	FILE	πy	GIU	<b>G11</b> .	100
					912					927					942			
	GAC	CAG	GGC	œ		TGG	GAA	AGG	GGC		CAC	œ	ATA	AAC		TAT	GAG	GTT
15			Gly															
	•	,						5				5						
		957					972					987				:	1002	
	ATG	AAG	$\infty$	$\infty$ A	GCA	GAA	GIG	GTG	$\infty$ T	GGG	CAC	CTC	ATC	ACA	ŒA	CIA	CTG	GAC
																		Asp
20		_								•					-			_
				L017					1032					L047				
																		CCT
	Thr	Arg	Leu	Val	His.	His	Αsη	.Val	Thr	Arg,	طتث	Glu	Thr.	Phe	Asp	Val	Ser	Pro
			-		•	-				•							•	
25	1062					L077					1092				-	1107		
																		GAG
	Ala	Val	Leu	Arg	Trp	Thr	Arg	Glu	Lys	Gln	Pro	Asn	Tyr	Gly	Leu	Ala	Ile	Glu
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	~		122				_	137				_	152				_	1167
30																		AGC
	Val	ши	urz	TEU	nis	ш	mr	Arg	шт	HIS	GIN	GIĀ	GIN	nis	vai	Arg	me	Ser.
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	~~~				182					197					1212	~~~	~~~	~
35			TTA															
	Arg	Ser	Leu	Pro	GII	GIĀ	Ser	GIY	ASN	JID	Αта	Gin	Leu	Arg	Pro	Leu	Leu	Val
	,	227				,	242				,	1257					1272	
			ccc	CATT	CAM			ccc	ന്മസ	ccc			CCA.	CCC	ccc			AAG
	Thr	Dho	Gly	Hic	yez Gerr	GOU	Am.	Glv	Hic	Ma	Ten	Thr	ATTT	Arm	Arror	Arro	Ala	Ivs
40		1110	GIJ	جسد	rsp	GI.	My	CLJ	*****	~~~				9	9			
			1	L287				1	L302				•	1317				
	-CCT	AGC	CCT		CAT	CAC	TCA			GCC	AGG	AAG			AAG	AAC	TGC	CCG
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5	1497 1512 1527 1542 GTC AAT TOO AGT ATC COO AAA GOO TGT TGT GTG COO ACT GAA CTG AGT GOO ATC Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
Ţ	1557 1572 1587 TOC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
10	1602 1617 (408) 1636 1646 1656 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG MET Val Val Glu Gly Cys Gly Cys Arg
15	1666 1676 1686 1696 1706 1716 1726 ATATACACAC CACACACACA CACCACATAC ACCACACACA
20	1736 1746 1756 1766 1776 1786 1796 ACAGACTGCT TCCTTATAGC TGGACTITTA TTTAAAAAAA AAAAAAAAAA AATGGAAAAA ATCCCTAAAC
25	1806 1816 1826 1836 1846 1856 1866 ATTCACCITG ACCITATITA TGACTITACS TGCAAATGIT TTGACCATAT TGATCATATA TTTTGACAAA
20	1876 1886 1896 1906 1916 1926 1936 ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAAA AAAAAAACT
30	1946 CTAGAGTOGA CGGAATTC,
35	wherein said process comprises the following steps:  a) screening of a gene library constructed from U-2 OS derived DNA or cDNA with a labelled bBMP-2 fragment by hybridization, b) isolating positive clones, and c) isolating the DNA-inserts from said clones.
40	12. The process according to claim 11, wherein the gene encodes human BMP-4 having the amino acid sequence given in claim 11.
45	13. A process for the preparation of a gene encoding a protein exhibiting properties of BMP-4 and comprising a DNA sequence:
	<ul> <li>a) which differs from a DNA sequence of claim 11 in codon sequence due to the degeneracy of the genetic code;</li> <li>b) which hybridizes with DNA sequence of claim 11 or section a), above; or</li> <li>c) represents a fragment, allelic or other variation of a DNA sequence of claim 11, whether said variation results</li> </ul>

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wherein said process comprises standard techniques of molecular biology.

in changes in the peptide sequence or not,

- 14. The process according to claim 13, wherein the DNA sequence is a genomic DNA sequence.
- 15. The process according to claim 13, wherein the DNA sequence is a cDNA sequence.
  - **16.** A vector containing the gene or DNA sequence prepared according to any one of claims 1 to 15 in operative association with an expression control sequence.

- 17. A cell transformed with a vector of claim 16.
- 18. The cell of claim 17 which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.
- 5 19. The cell of claim 18 which is a CHO cell.
  - 20. A process for the preparation of a protein exhibiting properties of BMP-2, wherein said process comprises the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence prepared according to any one of claims 1 to 10, and recovering said protein from said culture medium.
  - 21. A process for the preparation of a protein exhibiting properties of BMP-4, wherein said process comprises the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence prepared according to any one of claims 11 to 15, and recovering said protein from said culture medium.
  - 22. A process for producing a protein exhibiting properties of BMP-2 or BMP-4, comprising the steps of culturing in a suitable culture medium the cell of claim 17 and isolating said protein from said culture medium.
- 20 23. A process for the preparation of a pharmaceutical composition comprising combining the proteins prepared according to any one of claims 20 to 22, individually or in combination with a pharmaceutically acceptable vehicle.
  - 24. The process according to claim 23, wherein said pharmaceutical composition further comprises a matrix capable of delivering the composition to the site of the bone or cartilage defect and providing a structure for inducing bone or cartilage formation.
  - 25. The process according to claim 24, wherein said matrix comprises hydroxyapatite, collagen, polylactic acid or tricalcium phosphate.
- 30 26. Use of a protein prepared according to any one of claims 20 to 22, individually or in combination, for the preparation of a pharmaceutical composition for inducing bone or cartilage formation.

## Patentansprüche

Patentansprüche für folgende Vertragsstaaten : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Menschliches BMP-2 codierendes Gen, umfassend die nachfolgende DNA-Sequenz:

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,	CTC CTC Let Let 460 CCC CCC Ala Ala	E GGC GGC GGC GGC GGC GGC GGC GGC GGC GG	415 GGC Gly TCG Ser	GCG Ala GGC Gly	GCT Ala 475 CGC Arg	GGC Gly	CTC Leu TCA Ser 535	430 GIT Val TCC Ser	COG Pro CAG Gln	GAG GLU 490 CCC PTO	CIG Leu TCT Ser	GGC Gly GAC Asp 550 CAG	445 CCC Arg GAG Glu	AGG Arg GIC Val	AAG Lys 505 CIG Leu	AG GI In Va TTC Phe AGC Ser	GAG GAG GAG GAG GAG GAG
 30	CIC CIC Leu Leu 460 CCC CCC Ala Ala	ET VA	415 GGC Gly TGG Ser CGG Arg	GCC GLY CTG Leu 580	GCT Ala 475 CGC Arg CIC Leu	GGC Gly  CCC Pro	CTCA CTCA Ser S35 ATG	430 GIT Val TCC Ser TTC Phe	CCG Pro CAG Gln GGC Gly 595	GAG GLU 490 CCC PTO CIG Leu	CIG Leu TCT Ser AAA Lys	GGC Gly GAC Asp 550 CAG Gln	445 CGC Arg GAG Glu AGA Arg	AGG Arg GIC Val CCC Pro 610	AAG Lys 505 CIG Leu ACC	AG G. In Va TTC Phe AGC Ser CCC Pro	GAG GAG Glu 565 AGG Ser

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	2.	Gen	, das r	nensc	hliche	s BMF	-2 co	diert, c	das die	in Ar	spruc	h 1 an	géget	ene A	\minos	säures	eque	nz aufv	veist.	

- Gen, das menschliches BMP-2 codiert, das die in Anspruch 1 angegebene Aminosäuresequenz aufweist.
- 3. Gen, das ein Protein codiert, das Eigenschaften von menschlichem BMP-2 zeigt, und eine DNA-Sequenz umfaßt, die:
  - (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 1 unterscheidet;
  - (b) mit einer DNA-Sequenz nach Anspruch 1 oder nach vorstehendem Absatz (a) hybridisiert; oder
  - (c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 1 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht.
  - 4. DNA-Sequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie eine genomische DNA-Sequenz ist.
- 5. DNA-Sequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie eine cDNA-Sequenz ist.
  - 6. Rinder-BMP-2 codierendes Gen, umfassend die nachfolgende DNA-Sequenz:

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5	(1) GGC G	CAC H	GAT D	GGG G	15 AAA K	GGA G	CAC H	CCT P	CTC L	30 CAC H	AGA R	AGA R	GAA E	AAG K	45 CGG R
	CAA Q			CAC H	60 AAA K	CAG Q	CGG R	AAA K	CGC R	75 CTC L	aag K	TCC S	AGC S	TGT C	AAG K
10	AGA R	CAC H	CCT P	TTA L	105 TAT Y	GTG V	GAC D	TTC F	AGT S	120 GAT D	g <b>t</b> g V	GGG G	TGG W	AAT N	135 GAC D
15	TGG W	ATC I	GTT V	GCA A	150 CCG P	CCG P	GGG G		CAT H	165 GCC A	TTT	TAC Y	TGC C	CAT H	180 GGG G
20	GAG E	TGC C	CCT P	TIT F	195 CCC P	CTG L	GCC A	GAT D	CAC H	210 CTT L	AAC N	TCC S	ACG T	AAT N	225 CAT H
05	GCC A	ATT I	CTC V	CXA Q	240 ACT T	CTG L	GTC V	AAC N	TCA S	255 GTT V	AAC	TCT S	"AAG K	ATT I	270 CCC P
25	AAG K	GCA A	TGC C	TGT C	385 GTC V	CCA P	ACA T	GAG E	CTC L	300 AGC S		ATC	TCC S	ATG M	315 CTG L
<b>30</b>	TAC Y	CTI L	GAT D	GAG	330 AAT N	GAG	AAG K	GTG V	GTA V	345 . TTA L		AAC N	TAT	CAG O	360 GAC D
35		G GTT	GTC V	GAG E		TGI	GGG G	TGI	CGI	9) TAG	CACA	397 .GCA	TAAA		07 TA
40	TAAA		17 TA T	'ATA'		27 FA T	TAGA		7 .C AG	CAAA	44° LAAA		AAGT	457 TGAC	
45	ACTT		67 AT T	TCCC		77 SA A	GACT	48 TTAT		.TGGA		g AA	TGGA	507 GAAA	
	AAGA		17 .CA C	AGCI		27 ET G.	AAAA		7 A TI	TATA	541 TCT:		GAAA	557 AGAA	
50	GTTG		67 AA C	AAAT		77 PT A	ATCA	58 GAGA		TATT	,				

- 7. Gen, das Rinder-BMP-2 codiert, das die Aminosäuresequenz von Anspruch 6 enthält.
- 55 8. Gen, das ein Protein codiert, das Eigenschaften von Rinder-BMP-2 zeigt, und DNA-Sequenzen umfaßt, die:
  - (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 7 unterscheiden;

		(b) mit eir	ner DNA-Se	equenz nach Anspr	uch 7 oder nach vor	rstehendem Absatz	(a) hybridisieren; oder
5						A-Sequenz nach Ans sequenz führen oder	pruch 7 darstellen, unabhän- nicht.
3	9.	DNA-Sequent	z nach Ans	pruch 8, dadurch g	ekennzeichnet, daß	sie eine genomisch	e DNA-Sequenz ist.
	10.	DNA-Sequent	z nach Ans	pruch 8, dadurch g	ekennzeichnet, daß	sie eine cDNA-Sequ	uenz ist.
10	11.	Menschliches	BMP-4 cod	dierendes Gen, um	fassend die nachfol	gende DNA-Sequen	z:
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5		CGC	:CGC	110 TGC		TCC	12 GGCT		GTAT			TTGI		140 CCC		GGG.	150 ATT	
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15		CGC	TAC	260 TGC	-	GAC	27 CTAI		AGC		80 CC	GTAC		290 CAT		GAG	300 CAA	
		GCA	CTG	310 CTG		CTT	32 CCCI		GCCI		30 CA	GCA			TCA		350 PTG0	
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30	GGT Gly	417 AAC Asn	CGA.	ATG MET	CIG Leu	atg Met	432 GIC Val	GIT	TTA	TTA Leu	TGC	447 CAA Gln	GTC Val	CTG Leu	CTA Leu	GGA	462 GGC Gly	GCG Ala
;	AGC Ser	CAT His	GCT Ala	477 AGT Ser	TIG Leu	ATA Ile	CCT Pro	GAG Glu	492 ACG Thr	GGG Gly	AAG Lys	aaa Lys	aaa Lys	507 GTC Val	GCC Ala	GAG Glu	ATT Ile	CAG Gln
35	522 GGC Gly	CAC His	GCG Ala	GCA Gly	cjà ccy	537 CGC Arg	OGC Arg	TCA Ser	GGG Gly	CAG Gln	552 AGC Ser	CAT His	GAG Glu	CTC Leu	CIG Leu	567 CGG Arg	GAC Asp	TTC Phe
40	GAG Glu	GOG Ala	582 ACA Thr	CIT Leu	CTG Leu	CAG Gln	ATG MET	597 TTT Phe	GGG Gly	CTG Leu	OGC Arg	OGC Arg	612 CGC Arg	ccc Pro	CAG Gln	CCT Pro	AGC Ser	627 AAG Lys
45	AGT Ser	GCC Ala	GTC Val	ATT Ile	642	GAC Asp	TAC Tyr	ATG MET	CGG Arg	657 GAT Asp	CTT Leu	TAC Tyr	CGG Arg	CTT Leu	672 CAG Gln	TCT Ser	GGG Gly	GAG Glu
50	GAG Glu	687 GAG Glu	GAA Glu	GAG Glu	CAG Gln	ATC Ile	702 CAC His	AGC Ser	ACT Thr	GGT Gly	CTT Leu	717 GAG Glu	TAT Tyr	CCT Pro	GAG Glu	CGC Arg	732 CCG Pro	GCC Ala
	AGC Ser	CCC Arg	GCC Ala	747 AAC Asn	ACC	GIG Val	AGG Arg	AGC Ser	762 TTC Phe	CAC	CAC His	GAA Glu	GAA Glu	777 CAT His	CIG -Leu	GAG -Glu	AAC Asn	ATC Ile

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	CT Pro CT Pro GAC Asp ATG MET 106 GAI ANG Thr 106 ANG	CCA GGG Pro Gly  CCT GAG Pro Glu  GAC CAG Asp Gln  957 ATG AAG MET Lys  ACG ACT CAG GTC Ala Val  CTG ACT Val Thr  CCA TCG ATG Ser  1227 ACC TTT Thr Phe  CCT AGC ATG His  GCC GA ALa Pro  GCT GE	CCA GGG ACC Pro Gly Thr  852 CCT GAG AAC Pro Glu Asn  GAC CAG GGC Asp Gln Gly  957 ATG AAG CCG Thr Arg Leu  1062 GCG GTC CTT Ala Val Leu  1122 GTG ACT CAC Val Thr His  CGA TOG TTA Arg Ser Leu  1227 ACC TTT GGC Thr Phe Gly  CGT AGC CCT Arg Ser Pro  1332 CGC CAC TCG Arg His Ser  GCC CAC CCA Arg His Ser  GCT GAC CCT GAC CCA Ala Pro Pro  GCT GAC CCT GCT GAC CCT GCT GAC CCT GCT GAC CCT CCT	CCA CCC ACC CCC ACC CCC ACC CAC CAC CAC	CCA GGG ACC AGT GAA Pro Gly Thr Ser Glu  852  CCT GAG AAC GAG GTG Pro Glu Asn Glu Val  GAC CAG GGC CCT GAT Asp Gln Gly Pro Asp  957  ATG AAG CCC CCA GCA MET Lys Pro Pro Ala  1017  ACC AGA CTG GTC CAC Thr Arg Leu Val His  1062  GCG GTC CTT CCC TGG Ala Val Leu Arg Trp  1122  GTG ACT CAC CTC CAT Val Thr His Leu His  1182  CGA TCG TTA CCT CAA Arg Ser Leu Pro Gln  1227  ACC TTT GGC CAT GAT Thr Phe Gly His Asp  1287  CGT AGC CCT AAG CAT Arg Ser Pro Lys His  1332  CGC CAC TCG CTC TAT Arg His Ser Leu Tyr  1392  GCC CCA CCA GGC TAC Ala Pro Pro Gly Tyr  149  GCT GAC CAC CTC AAC  149	CCA GGG ACC AGT GAA AAC Pro Gly Thr Ser Glu Asn  852  CCT GAG AAC GAG GTG ATC Pro Glu Asn Glu Val Ile  GAC CAG GGC CCT GAT TGG Asp Gln Gly Pro Asp Trp  957  ATG AAG CCC CCA GCA GAA MET Lys Pro Pro Ala Glu  1017  ACC AGA CTG GTC CAC CAC Thr Arg Leu Val His His  1062  GTG ACT CAC CTC CAT CAG Ala Val Leu Arg Trp Thr  1122  GTG ACT CAC CTC CAT CAG Val Thr His Leu His Gln  1182  CGA TGG TTA CCT CAA GGG ATG STA CTT CAC Arg Ser Leu Pro Gln Gly  1227  ACC TTT GGC CAT GAT GGC Thr Phe Gly His Asp Gly  1287  CGT AGC CCT AAG CAT CAC Arg Ser Pro Lys His His  1332  GTG ACT CAC GCC TC TAT GTG ATG His Ser Leu Tyr Val  1392  GCC CCA CCA GGC TAC CAG Ala Pro Pro Gly Tyr Gln  1452  GCT GAC CAC CTC AAC TC	CCA GGG ACC AGT GAA AAC TCT Pro Gly Thr Ser Glu Asn Ser Glu Asn Glu Val IIe Ser Glu Asn Glu Val IIe Ser Glu Asn Glu Val IIe Ser GAC CAG GGC CCT GAT TGG GAA Asp Gln Gly Pro Asp Trp Glu Pro Asp Glu Val Glu Val III GGC CAC CAC AAT Thr Arg Ieu Val His His Asn III Arg Ieu Val His His Asn III Arg Ieu Arg Trp Thr Arg III Ar	CCA GGG ACC AGT GAA AAC TCT GCT Pro Gly Thr Ser Glu Asn Ser Ala  852  CCT GAG AAC GAG GTG ATC TCC TCT Pro Glu Asn Glu Val Ile Ser Ser  GAC CAG GGC CCT GAT TGG GAA AGG Asp Gln Gly Pro Asp Trp Glu Arg  957  ATG AAG CCC CCA GCA GAA GTG GTG MET Lys Pro Pro Ala Glu Val Val Val 1017  ACG AGA CTG GTC CAC CAC AAT GTG Thr Arg Leu Val His His Asn Val 1062  1062  1077  GCG GTC CTT CGC TGG ACC CGG GAG Ala Val Leu Arg Trp Thr Arg Glu 1137  GTG ACT CAC CTC CAT CAG ACT CGG Val Thr His Leu His Gln Thr Arg Clu 1182  CGA TGG TTA CCT CAA GGG AGT GGG AAG TGG GTG TTA CCT CAA GGG AGT GGG AAC TTT GGC CAT GAT GGC CGG GGC TTT GGC CAT GAT GGC CGG GGC TTT GGC CAT GAT GGC CGG GGC TTT AGC TCT AAG CAT CAC TCA CAG ATG GGC ATG ATG GGC CAC TTT GGC CAT CAT GAT GGC CGG GGC TTT AGC CAC TGG CAC TCA CAG ATG SEP Pro Lys His His Ser Gln 1332  1287  CGT AGC CCT AAG CAT CAC TCA CAG ATG SEP Pro Lys His His Ser Gln Ala Pro Pro Glv Tvr Gln Ala Phe 1452  GCT GAC CAC CTC TAT CAC TCA ACC ACC AAG ATG His Ser Leu Tyr Val Asp Phe	CCA GGG ACC AGT GAA AAC TOT GCT TITT Pro Gly Thr Ser Glu Ash Ser Ala Phe  852 CCT GAG AAC GAG GTG ATC TCC TOT GCA Pro Glu Ash Glu Val Ile Ser Ser Ala  912 GAC CAG GGC CCT GAT TGG GAA AGG GGC ASp Gln Gly Pro Asp Trp Glu Arg Gly  957 ATG AAG CCC CCA GCA GAA GTG GTG CCT MET Lys Pro Pro Ala Glu Val Val Pro  1017 1032 ACG AGA CTG GTC CAC CAC AAT GTG ACA Thr Arg Leu Val His His Ash Val Thr  1062 1077 GCG GTC CTT CCC TGG ACC CGG GAG AAG Ala Val Leu Arg Trp Thr Arg Glu Lys  1122 GTG ACT CAC CTC CAT CAG ACT CGG ACC Val Thr His Leu His Gln Thr Arg Thr  1182 CGA TCG TTA CCT CAA GGG AGT GGG AAT Arg Ser Leu Pro Gln Gly Ser Gly Ash  1227 1242 ACC TTT GGC CAT GAT GGC GGC CAT Thr Phe Gly His Asp Gly Arg Gly His  1287 1287 1302 CGT AGC CCT AAG CAT CAC TCA CAG GGG AAT ATG THR Phe Gly His Asp Gly Arg Gly His  1332 1332 1347 CGC CAC TCG CTC TAT GTG GAC TTC AGC ATG His Ser Leu Tyr Val Asp Phe Ser  1392 1407 GCC CAC CCA CGC TAC CAG GCC TTC TAC AAC AAC CAG CAG AAG AAC AAC CTC AAC CAG CGC TTC TAC AAC AAC CTC CAA CAA CAC CTC AAC CTC AAC CAG CGC TTC TAC AAC AAC CTC CAA CAA CAC CTC AAC CAG GCC TTC TAC AAC AAC CTC CAA CCA CCA CCA C	CCA GGG ACC AGT GAA AAC TOT GCT TIT GGT PTO Gly The See Glu Ash See Ala Pie Arg  852  CCT GAG AAC GAG GTG ATC TOC TOT GCA GAG PTO Glu Ash Glu Val Ile See See Ala Glu  GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC Asp Gln Gly PTO Asp Trp Glu Arg Gly Phe  957  ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG MET Lys PTO PTO Ala Glu Val Val PTO Gly  ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG The Arg Leu Val His His Ash Val The Arg  1062  1077  GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG Ala Val Leu Arg Trp The Arg Glu Lys Gln  1122  GTG ACT CAC CTC CAT CAG ACT CGG ACC CAC Val The His Leu His Gln The Arg The His  1182  CGA TGG TTA CCT CAA GGG AGT GGG AAT TGG ACT TTA CCT CAA GGG AGT GGG AAT TGG ACT TTA CCT CAA GGG AGT GGG AAT TGG ACT TTA CCT CAA GGG AGT GGG AAT TGG ACT TTA CCT CAA GGG AGT GGG AAT TGG ACT TTA CCT CAA GGG ACT CGG ACT CGC The Phe Gly His Asp Gly Arg Gly His Ala  1287  1227  1242  ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC The Phe Gly His Asp Gly Arg Gly His Ala  1287  1332  1287  1332  1347  CGC CAC TGG CTC TAT GTG GAC TTC AGC GAT Arg His See Leu Tyr Val Asp Phe See Asp  1392  1497  GCC CCA CCA CGC TAC CAG CGC TTC TAC TGC Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys  1452  1452  1452  1466  CCT GAC CAC CTC AAC CTC AAC TCA ACC AAC CAT GCC CAT GAC CAC CTC AAC TCA CTC AAC CAT GCC Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys  1452  1452  1452  1467	CCA GGG ACC AGT GAA AAC TCT GCT TIT CGT TTC Pro Gly Thr Ser Glu Ash Ser Ala Phe Arg Phe  852  CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT Pro Glu Ash Glu Val Ile Ser Ser Ala Glu Leu  912  GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His  957  ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC MET Lys Pro Pro Ala Glu Val Val Pro Gly His  1017  1032  ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG Thr Arg Leu Val His His Ash Val Thr Arg Trp  1062  1077  GCG GTC CTT CGC TGG ACC CGG GAG AAG CAG CCA Ala Val Leu Arg Trp Trr Arg Glu Lys Gln Pro  1122  GTG ACT CAC CTC CAT CAG ACT CGG AAC CAC CAG Ala Val Leu Arg Trp Trr Arg Glu Lys Gln Pro  1122  GTG ACT CAC CTC CAT CAG ACT CGG AAC CAC CAG Arg Ser Leu Pro Gln Gly Ser Gly Ash Trp Ala  1227  1242  ACC TTT GGC CAT GAT GGG GGC CAT GCC TTG Thr Phe Gly His Asp Gly Arg Gly His Ala Leu  1287  1287  1302  GT AGC CCT AAG CAT CAC TCA CAG GGG GAC AAC Arg Ser Pro Lys His His Ser Gln Arg Ala Arg  1332  1347  1362  GCT AGC CAC TCC TAT GTG GAC TTC AGC GAT GTG Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val  1392  1497  GCC CCA CCA CCC TCA CAC GCC TTC TAC TGC CAT Ala Pro Pro Glv Tvr Gln Ala Phe Tvr Cvs His  1452  1452  1467  GCT GAC CAC CTC AAC CTC AAC TCA ACC CAT GCC AI	CCA GGG ACC AGT GAA AAC TOT GCT TIT GGT TTC CTC PTO Gly The See Glu Ash See Ala Pie Arg Pie Leu  852  857  CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG PTO Glu Ash Glu Val Ile See See Ala Glu Leu Arg  912  GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC CGT Asp Gln Gly PTO Asp TTp Glu Arg Gly Pie His Arg  957  ATG AAG CCC CCA GCA GAA GTG GTG CTT GGG CAC CTC MET Lys PTO PTO Ala Glu Val Val PTO Gly His Leu  1017  ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA The Arg Leu Val His His Ash Val The Arg Trp Glu  1062  1077  1092  GCG GTC CTT CCC TGG ACC CGG GAG AAG CAC CAC CAA Ala Val Leu Arg Trp The Arg Glu Lys Gln PTO Ash  1122  TIB ACT CAC CTC CAT CAG ACT CGG ACC CAC GAG CAA ACC Val The His Leu His Gln The Arg The His Gln Gly  1182  CGA TGG TTA CCT CAA GGG AGC GAA ATT TGG GCC CAG Arg See Leu PTO Gln Gly See Gly Ash Trp Ala Gln  1227  1242  1257  ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TGG ACT TTT GGC CAT GAT GGC CGG GGC CAT GCC TTT GGC CAT GAT GGC CGG GGC CAT GCC The Pie Gly His Asp Gly Arg Gly His Ala Leu The  1287  1302  CGT AGC CCT AAG CAT CAC TCA CAG GGC CAT GCC Arg See PTO Lys His His See Gln Arg Ala Arg Lys  1332  1247  1362  137  1362  CGC CAC TGC CTC TAT GTG GAC TTC AGC GAT GGC Arg His See Leu Tyr Val Asp Pie See Asp Val Gly  1392  1457  GCC CAA CCA GGC TAC CAG GCC TTC TAC TGC GAT GGC Ala PTO PTO Glv Tvr Gln Ala Pie Tvr Cvs His Glv  1452  1452  1457  CCC CAC CAC CCC CAC CTC AAC CAG CCC TTC TAC TGC CAT GGC Ala PTO PTO Glv Tvr Gln Ala Pie Tvr Cvs His Glv	CCA GGG ACC AGT GAA AAC TOT GCT TIT GGT TTC CTC TITP Pro Gly Thr Ser Glu Ash Ser Ala Phe Arg Phe Leu Phe  852  CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC Pro Glu Ash Glu Val Tle Ser Ser Ala Glu Leu Arg Leu Ash Glu Ash Glu Val Tle Ser Ser Ala Glu Leu Arg Leu GAC CAG GGC CTC GAT TGG GAA AGG GGC TTC CAC CGT ATA Ash Gln Gly Pro Ash Trp Glu Arg Gly Phe His Arg Tle 957  ATG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Tle 1017  ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TAT Arg Leu Val His His Ash Val Thr Arg Trp Glu Thr 1062  1077  1062  1077  1092  GCG GTC CTT CCC TGG ACC CGG GAG AAG CAG CCA AAC TAT Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Ash Tyr 1122  1127  1122  1137  1152  GTG ACT CAC CTC CAT CAG ACT GGG AAC CAC CAG GGC CAG Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln 1182  1182  1197  GCA TGG TTA CCT CAA GGG ACT GCG CAT GCC TTG ACC CAG GGC CAG CTC Arg Ser Leu Pro Gln Gly Ser Gly Ash Trp Ala Gln Leu 1227  ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA ATG CTC Arg Ser Leu Pro Gln Gly Ser Gly Ash Trp Ala Gln Leu 1227  ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA ATG CTC Arg Ser Leu Pro Gln Gly Ser Gly Ash Trp Ala Gln Leu 1227  ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA ATG CTC Arg Ser Leu Pro Gln Gly Ser Gly Ash Trp Ala Gln Leu 1227  ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA ATG CTC AAG CAT CAC CAG GGC CAG CAG CAG CAG CAG CAG CAG	CCA GGG ACC AGT GAA AAC TOT GCT TIT GGT TITC CTC TIT AAC Pro Gly Thr Ser Glu Ash Ser Ala Phe Arg Phe Leu Phe Ash 852  CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT GGG CTC TTC Pro Glu Ash Glu Val I le Ser Ser Ala Glu Leu Arg Leu Phe Ash Ash Glu Gal Ash Glu Val I le Ser Ser Ala Glu Leu Arg Leu Phe Ash Glu Gac CAG GGC CTT GTT TAG GAA AGG GGC TTC CAC GGT ATA AAC Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg I le Ash 957  ATG AAG CCC CCA GCA GCA GAG GTG GTG CCT GGG GAC CTC ATC ACA MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu I le Thr Arg Leu Val His His Ash Val Thr Arg Trp Glu Thr Phe 1062  1007  ACG AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT Thr Arg Leu Val His His Ash Val Thr Arg Trp Glu Thr Phe 1062  1077  CCG GTC CTT CCC TGG ACC CGG GAG AAG CAG CCA AAC TAT GGG Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Ash Tyr Gly 1122  CTG ACT CAC CTC CAT CAG ACT CGG AAC CAC CAG GGC CAG CAT Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His 1182  CGA TGG TTA CCT CAA GGG AGT GGG AAC CAC CAG GGC CAG CAT Val Thr His Leu Pro Gln Gly Ser Gly Ash Trp Ala Gln Leu Arg 1227  ACC TTT GGC CAT GAT GGC GGG GGC CAT GCC TTG ACC CAG GGC CAG CAT CAC TTG ACC CAT GGC ATT GGG ACC CAT GGC TTG ACC CAT GAT GGG AGC TTG ACC CAT GAT GGG AGC TTG ACC CAT GAT GGC CAT GGC TTG ACC CAT GGC TTG ACC CAT GGC TTG ACC CAT GGC CAT GGC TTG ACC CAT GGC CAT GGC TTG AAC ACT TTT AGC CAT GGC TTG AAC ACT TTT AGC CAT GGC TTG AAC ACT TTT AGC CAT GGC TTG ACC CAT GGC TTG AAC ACT TTT AGC GAC CAT GGC TTG AAC ATT GTG CAC TTC TAC GGC CAC GGC TTG AAT ATT GTG CAC TTC TAC TTG CAC GGC TTG AAC AAC AAC AAC AAC AAC AAC AAC AAC AA	CCA GGG ACC AGT GAA AAC TCT GCT TTT GGT TTC CTC TTT AAC CTC Pro Gly Thr Ser Glu Ash Ser Ala Phe Arg Phe Leu Phe Ash Leu Pro Gly Thr Ser Glu Ash Ser Ala Phe Arg Phe Leu Phe Ash Leu Room Ser Gly Thr Ser Glu Ash Ser Ala Phe Arg Phe Leu Phe Ash Leu Room Ser Gly Ash Gly Ash Gly Arc TCC TCT GGA GAG CTT GGG CTC TTC GGG AAC GAG GGC GTT GGG GGG GTG ATC TCC GGG GAC GTT GGG GGC GTT GGG GAA AGG GGC TTC CAC GGT ATA AAC ATT Asp Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Ile Ash Ile 957  ANG AAG CCC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA GGA MET Lys Pro Pro Ala Glu Val Val Pro Gly His Leu Ile Thr Arg 1017  ACC AGA CTG GTC CAC CAC AAT GTG ACA CGG TGG GAA ACT TTT GAT Thr Arg Leu Val His His Ash Val Thr Arg Trp Glu Thr Phe Asp 1062  1077  GCG GTC CTT CCC TGG ACC CGG GAG AAG CAG CCA AAC TAT CGG CTA Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Ash Tyr Gly Leu Ala Val Leu Arg Trp Thr Arg Glu Lys Gln Pro Ash Tyr Gly Leu Ile Acc CTC CAC CAC CAC ACC GGG CAC CAC CAC CAC CA	CCA GGG ACC AGT GAA AAC TCT GCT TTT GGT TTC CTC TTT AAC CTC AGC PTO Gly The Ser Glu Ash Ser Ala Phe Arg Phe Leu Phe Ash Leu Ser  852  857  CCT GAG AAC GAG GTG ATC TCC TCT GCA GAG CTT CGG CTC TTC CGG GAG PTO Glu Ash Glu Val Ile Ser Ser Ala Glu Leu Arg Leu Phe Arg Glu  GAC CAG GGC CCT GAT TGG GAA AGG GGC TTC CAC GGT ATA AAC ATT TAT ASP Gln Gly PTO ASP Trp Glu Arg Gly Phe His Arg Ile Ash Ile Tyr  957  ATC AAG CCC CA GCA GAA GTG GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA MET Lys PTO PTO Ala Glu Val Val PTO Gly His Leu Ile The Arg Leu  1017  ACG AGA CTG GTC CAC CAC AAT GTG GTG ACA CGG TGG GAA ACT TTT GAT GTG TATA ATG Leu Val His His Ash Val The Arg Tp Glu The Phe Asp Val  1062  1077  GCG GTC CTT CCC TGG ACC CGG GAA AAG CAG CAC AAC TAT GGG CTA GCC Ala Val Leu Arg Trp The Arg Glu Lys Gln PTO Ash Tyr Gly Leu Ala  1122  1137  1152  GTG ACT CAC CTC CAT CAG ACT CGG AAC CAC CAG GGC CAG CAT GTC AGG Val The His Leu His Gln The Arg The His Gln Gly Gln His Val Arg  1182  CGA TCG TTA CCT CAA GGG ACT CGG AAT TGG GCC CAG CTC CAG CAC CTC Arg Ser Leu PTO Gln Gly Ser Gly Ash Trp Ala Gln Leu Arg PTO Leu  1227  ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGG ACG TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CTC Arg Ser Leu PTO Gln Gly Ser Gly Ash Trp Ala Gln Leu Arg PTO Leu  1227  ACC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC TTT FE GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC TTT FE GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC TTT GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC TTT FE GGC CAT GAT GGC CGG GGC CAT GCC TTG ACC CGA CGC TTT FE GGC CAT GAT GGC CGC GGC CAT GCC TTG ACC CGA CGC TTT ACC CTC CAT GGC CGC CGC CAT GCC TTG ACC CTC CGG CCC TTT ACC CTC CAT GGC CAC TTC ACC CGG CCC CTC CGC CCC CTC CTC  CGT AGC CAT GCC TAC CAC CAC TTC ACC CGG GCC TTC TAT GTG CAC CTC CTC  ATG CCC CAC CCC CCC CAC CAC CCC TTC TAC TGC CAC CTC CCC CTC TTT  Ala PTO PTO Glv Tvr Gln Ala Phe Se	CCA GGG ACC AGT GAA AAC TOT GCT TIT GGT TTC CTC TTT AAC CTC AGC AGC Pro Gly Thr Ser Glu Ash Ser Ala Phe Arg Phe Leu Phe Ash Leu Ser Ser B852 CCT GGG AAC GAG GGG ATC TCC TCT TCC GGG GAG CAG CTC TTC CGG GAG CAG CTC TTC CGG GAG CAG CTC GGG CAG CAC CTC GGG AAC GAG GGG AAC CAG GAG GGG AAC GAA AGG GGC TTC CAC CGT ATA AAC ATT TAT GAG ASP Gln Gly Pro Asp Trp Glu Arg Gly Phe His Arg Tle Ash Tle Tyr Glu Psp Ash CCC CAA GCA GAA GGG GCT GGG CAC CTC ATC ACA CGA CTA CTA CAC AGA GTA GTA AAG ACC CCA GCA GAA GTG GTG CCT GGG CAC CTC ATC ACA CGA CTA CTA AAC AGA CTA CTA CAC AGA CAC CAC CAC CAC CAC CAC CAC CA

5	1497 1512 1527 1542 GTC AAT TOO AGT ATC COO AAA GOO TGT TGT GTG COO ACT GAA CTG AGT GOO ATC Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
10	1557 1572 1587  TOO ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu  1602 1617 (408) 1636 1646 1656
	ATG GTA GTA GAG GGA TGT GGG TGC GGC TGAGATCAGG CAGTCCTTGA GGATAGACAG MET Val Val Glu Gly Cys Gly Cys Arg
15	
	1666 1676 1686 1696 1706 ATATACACAC CACACACA CACCACATAC ACCACACACA
20	1716 1726 1736 1746 1756
	TCCACTCACC CACACACTAC ACAGACTGCT TCCTTATAGC TGGACTTTTA
	1766 1776 1786 1796 1806
	TTTAAAAAA AAAAAAAAA AATGGAAAAA ATCCCTAAAC ATTCACCTTG
25	1816 1826 1836 1846 1856
	1816 1826 1836 1846 1856 ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA
	noorman tonorrano tonament remonstration
30	1866 1876 1886 1896 1906
30	TTTTGACAAA ATATATTAT AACTACGTAT TAAAAGAAAA AAATAAAATG
	1916 1926 1936 1946
	AGTCATTATT TTAAAAAAAA AAAAAAAACT CTAGAGTCGA CGGAATTC
35	
	12. Gen, das menschliches BMP-4 codiert, das die in Anspruch 11 angegebene Aminosäuresequenz aufweist.
	13. Gen, das ein Protein codiert, das Eigenschaften von BMP-4 zeigt, und eine DNA-Sequenz umfaßt, die:
40	(a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-sequenz nach Anspruch 11 unterscheidet;
	(b) mit einer DNA-Sequenz nach Anspruch 11 oder nach vorstehendem Absatz (a) hybridisiert; oder
45	(c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 11 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht.
	14. DNA-Sequenz nach Anspruch 13, dadurch gekennzeichnet, daß sie eine genomische DNA-Sequenz ist.
50	15. DNA-Sequenz nach Anspruch 13, dadurch gekennzeichnet, daß sie eine cDNA-Sequenz ist.
	<b>16.</b> Vektor, enthaltend das Gen oder die DNA-Sequenz nach einem der Ansprüche 1 bis 15 in einer funktionellen Verbindung mit einer Expressions-Kontrollsequenz.
55	17. Zelle, dadurch gekennzeichnet, daß sie mit einem Vektor nach Anspruch 16 transformiert ist.

18. Zelle nach Anspruch 17, dadurch gekennzeichnet, daß sie eine Säugerzelle, eine Bakterienzelle, eine Insekten-

zelle oder eine Hefezelle ist.

- 19. Zelle nach Anspruch 18, dadurch gekennzeichnet, daß sie eine CHO-Zelle ist.
- Protein, das Eigenschaften von BMP-2 aufweist, das durch ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 1 bis 10 codiert ist.

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- 21. Protein, das Eigenschaften von BMP-2 aufweist, das erhältlich ist durch die Schritte
  - Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Vektor ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 1 bis 10 umfaßt, und

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Gewinnen des Proteins aus dem Kulturmedium.

22. Protein, das Eigenschaften von BMP-4 aufweist, das durch ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 11 bis 15 codiert ist.

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- 23. Protein, das Eigenschaften von BMP-4 aufweist, das erhältlich ist durch die Schritte
  - Züchten einer mit einem Expresionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Vektor ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 11 bis 15 umfaßt und

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- Isolieren des Proteins aus dem Kulturmedium.
- 24. Verfahren zur Herstellung des Proteins nach Anspruch 21 oder 23, umfassend die Schritte

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- Züchten der Zelle nach Anspruch 17 in einem geeigneten Kulturmedium und
- Gewinnen des Proteins aus dem Kulturmedium.
- 25. Arzneimittel, dadurch gekennzeichnet, daß es, einzeln oder in Kombination, die Proteine nach einem der Ansprüche 20 bis 23 und einen pharmakologisch verträglichen Träger umfaßt.
  - 26. Arzneimittel nach Anspruch 25, dadurch gekennzeichnet, daß es ferner eine Matrix umfaßt, die fähig ist, das Arzneimittel an die Stelle des Knochen- oder Knorpelschadens zu liefern und eine Struktur zur Induktion der Knochen- oder Knorpelbildung bereitzustellen.

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- Arzneimittel nach Anspruch 26, dadurch gekennzeichnet, daß die Matrix Hydroxyapatit, Kollagen, Polyessigsäure oder Tricalciumphosphat umfaßt.
- 28. Verwendung des Proteins nach einem der Ansprüche 20 bis 23, einzeln oder in Kombination, zur Herstellung eines Arzneimittels zur Induktion der Knochen- oder Knorpelbildung.

#### Patentansprüche für folgenden Vertragsstaat : AT

45 1. Verfahren zur Herstellung eines menschliches BMP-2 codierenden Gens, das die nachfolgende DNA-Sequenz umfaßt:

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	GTCGAC				rgTG				GG		GGA			'GAA	CTTC		
5	CAGGGA	6 GAAT	_	 CTTC		O A C	CCCI		80 TG	CGCC	GGT	90 GCC	ттт	GCC	100 CCAC		
	CGGAGO	110 CTGC		CGCC		_	CGAC	_	30 CA	CCGC		140 TCC		CCT	150 CGG(	-	
10	CTTGC	160 CCGAC		rgac	17 SACG		GTT		8 0 GC	GTG <i>P</i>		190 GAG		CTG	200 CGC0		
15	GCCGG	210 CACCC		GAG <i>I</i>	22 AAGG		GAG		30 AG	AAA		240 ACG		:ATT	250 CGG1	-	
	CCTTG	260 CGCCA		rcci	27 TTTG		CAG		80 TT	TCC!	\TGT	290 GGA		TCT	300 TTC		
20	ATGGA	310 CGTGT		CCG	32 CGTG		TCT:		30 CG	GACT	rgcg	340 GTC		TAA	350 <b>AGG</b> 1		
25	CGACC A	1) TG GT ET Va	G GO 1 Ala	c co a Gl	37 35 AC 37 Th	$\dot{x} \alpha$	ed c) ec 10	sic /s L	en P L C	IA GO	35 S T la L	e c	<i>ia</i> 14	en e	x c	oo YG G Ln Va	er IC
	CTC CTC	GGC	415 GGC ( Gly )	GCG Ala	GCT Ala	GGC Glv	CTC	430 GTT Val	ccc	GAG Glu	CIG Leu	GGC G1v	445 CGC	AGG	AAG	TIC	GCG
30	460 cos cos Ala Ala	TCG '	TCS (	GGC	475 CGC	œ	TCA	TCC	CAG	490 ccc	TCT	GAC	GAG	ണ്ട	505 CTG	AGC	GAG
35	TTC GAG Phe Glu	520 TTG	œ .	cre	crc	AGC	535 ATG	TTC	GGC	crg	AAA	550 CAG	AGA	<u></u>	ACC	ಞ	565 AGC
40	yrd yzi yce cyc	: cc	GIG (	580 GTG	$\infty$	$\infty$	TAC	ATG	595 CTA	GAC	CTG	TAT	œc	610 AGG	CAC	TOG	GGT

	CAG Gln	625 CCG Pro	GGC Gly	TCA Ser	ccc Pro	GCC Ala	640 CCA Pro	GAC Asp	CAC His	CGG Arg	TTG Leu	GAG Glu	AGG Ar <del>u</del>	GCA Ala	GCC Ala	AGC Ser	670 CGA Arg	GCC
5	AAC	ACT	GIG	685 CGC	AGC	TTC	CAC	CAT	700 GAA	gaa	TCT	TTG	ĠĀĀ	715 GAA	CIA	CCA	GAA	ACG
10	730	ınr	vaı	Arg	Ser	745	His	Fis	Glu	Glu	Ser 760		Glu	Glu	Leu	Pro 775	Glu	Thr
	AGT	GCG Gly	aaa Lys	ACA Thr	ACC Thr	ŒĢ	AGA Arg	TIC Phe	TIC Phe	TTT Phe	AAT	TIA	AGT Ser	TCT Ser	ATC Ile	$\infty$	ACG Thr	GAG Glu
15	GAG Glu	TTT Phe	790 ATC Lle	ACC Thr	TCA Ser	GCA Ala	GAG Glu	805 CIT Leu	GLn	GTT Val	TTC	CGA Arg	820 GAA Glu	eju Cæ	ATG MET	CAA Gln	GAT Asp	835 GCT Ala
20	TTA Leu	GJA GGY	AAC Asn	AAT Asn	850 AGC Ser	AGT	TTC Phe	CAT His	CAC His	865 CGA Arg	ATT	AAT ASD	ATT	TAT Tyr	880 GAA Glu	ATC	ATA Ile	AAA Lys
	CCI Pro	895 GCA Ala	ACA	GCC	AAC Asn	TOS Ser	910 AAA Lys	TIC	e coo	GIG Val	ACC Thr	925 AGT Ser	CIT	TIG Leu	GAC Asp	ACC	940 AGG Arg	TIG
25					GCA					AGI					$\infty$			ATG MET
30	100 CGG Arg	TCC	ACI Thr	Ala	Glr	Gly	CAC His	ala s	L AST	ı His	Gly	TIC	. Val	Val	. Glu	1045 GIG Val	GCC	CAC His
35	TIG	GA(	1060 GAC	AA	A CAZ	. cci	GIO	1075	S S AAC	AG?	A CAI	rgn	1090 ' AGC	) S ATP	, ago	AGG Arc	; TCI ; Ser	1105 TIG
						AGC					AG(					YC]		GCC Gly
40			r GGJ					CIX					AA					A CAC S His
45	AAA	CA(	G CCC	1225 3 AA	A CGG	CII	r aad	G TO	1240 2 AG	C TG	r aa	G AGI	A CAG	1255 C CC:	TT T	G TAC	CIC	GAC LAsp
	12 TT	70 C AC	T GA	್ ಆ	.c cc	128 G TG	5 G Aa	E T	ic Ta	g ai	130 T G	00 0G GC	T CC	$x \propto$	<b>%</b> GG	131 G TA	.5 IT CA	ac GCC s Ala
50	TI	T	133 C TC	10 3C CI	AC GC	iA Gi	n a	134 3C Q	45 Or T	r c	II (	ig go	130 II G	60 HT C	ಷ್ ೧	ಣ ಸ	ac I	1375 CC ACT er Thr
55	22 25	I C	er Go	x xi la I!	139 TT GT Le Va	r c	ag ak Ln T	og M og L	ng G	140 IC A El A	C T	CT G er Va	M A	AC IX SII S	14: CT Al er L	AG A	TT C le P	CT AAG

1435 1450 1465 1480 GCA TGC TGT GTC COG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu 5 1510 1525 AAT GAA AAG GIT GIA TIA AAG AAC TAT CAG GAC ATG GIT GIG GAG GGT TGT GGG Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly 10 1540 (396) 1553 1563 1573 1583 1593 1603 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTITTAG AAAAAAGAAA Cys Arg 15 AAAA, wobei das Verfahren die nachfolgenden Schritte umfaßt: (a) Absuchen einer Genbank durch Hybridisieren mit einem markierten bBMP-2-Fragment, wobei die Genbank 20 aus einer von U-2 OS abgeleiteten DNA oder cDNA konstruiert war, (b) Isolieren positiver Clone und (c) Isolieren der DNA-Insertionen aus diesen Clonen. 25 Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das Gen menschliches BMP-2 codiert, das die in Anspruch 1 angegebene Aminosäuresequenz aufweist. Verfahren zur Herstellung eines Gens, das ein Protein codiert, das Eigenschaften von menschlichem BMP-2 zeigt, 30 und eine DNA-Sequenz umfaßt, die: (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 1 unterscheidet; (b) mit einer DNA-Sequenz nach Anspruch 1 oder nach vorstehendem Absatz (a) hybridisiert; oder 35 (c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 1 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht, 40 wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt. 4. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist. 5. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist. 45 6. Verfahren zur Herstellung eines Rinder-BMP-2 codierenden Gens, umfassend die nachfolgende DNA-Sequenz: 50

```
30
                       15
       GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG
                     X
                         G
                                             3
                                                             90
                       60
       CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG
                             3
10
                                         120
       AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC
                             D
                                         155
                      150
15
       TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT GGG
                          P
                             G
                                 Y
                                         .210
                                                            225
                      195
       GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT
20
          С
              P
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                          L
                              Α
                                     H
                                        L
                                             N
                  F
                                         255
                      240
       GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT
                          L
                             V
                                 N
                                     S
                                             N
25
              300
       AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG
                      v
                          5
                                  Ξ
                                         S
30
                                          345
                      330
        TAC CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC
                      N
                                  V
                                      V
                                             К
                                                           407
                      375 ·
                                      (129)
                                                397
35
        Ξ
                                  C
                                      2
                      G
                          С
                              G
40
                                              447
                                                         457
              417
                         427
                                    437
        TAAATATATA TATATATATA TTAGAAAAAC AGCAAAAAAA TCAAGTTGAC
                                              497
                         477
                                    487
        ACTITAATAT TICCCAATGA AGACTITATI TATGGAATGG AATGGAGAAA
45
                                                         557
              517
                         527
                                    537
                                               547
        AAGAAAACA CAGCTATTTT GAAAACTATA TTTATATCTA CCGAAAAGAA
                         577
                                    587
50
        GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT
```

wobei das Verfahren die nachfolgenden Schritte umfaßt:

- (a) Absuchen einer Genbank mit einer markierten auf der Grundlage der Aminosäuresequenz eines Fragmentes von bBMP-2 entworfenen Sonde, wobei die Genbank aus Rinderleber-DNA oder cDNA konstruiert wurde,
- (b) Isolieren positiver Clone und

- (c) Isolieren der DNA-Insertionen aus diesen Clonen.
- 7. Verfahren nach Anspruch 6, dadurch gekennzeichnet, daß das Gen Rinder-BMP-2 codiert, das die Aminosäuresequenz von Anspruch 6 aufweist.
- 8. Verfahren zur Herstellung eines Genes, das ein Protein codiert, das Eigenschaften von Rinder-BMP-2 zeigt, und DNA-Sequenzen umfaßt, die:
  - (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 7 unterscheiden;
    - (b) mit einer DNA-Sequenz nach Anspruch 7 oder nach vorstehendem Absatz (a) hybridisieren; oder
- (c) Fragmente, allelische oder andere Variationen einer DNA-Sequenz nach Anspruch 7 darstellen, unabhängig davon, ob die Variationen zu Änderungen in der Peptidsequenz führen oder nicht,

wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt.

- 9. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist.
- 10. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist.
- 11. Verfahren zur Herstellung eines menschliches BMP-4 codierenden Genes, das die nachfolgende DNA-Sequenz umfaßt:

	10	20	30	40	50
			GGGAGGGAGG	GAAGGAGCGC	GGAGCCCGGC
		70	80	90	100
30	60 CCGGAAGCTA	70 GGTGAGTGTG	GCATCCGAGC		<del>-</del>
		120	130	140	150
	110 CGCCGCTGCT	GCTCCGGCTG	AGTATCTAGC		GATGGGATTC
35	160	170	180	190	200
	CCGTCCAAGC	TATCTCGAGC			GCCCTCGCCC
	210	220	230		
40	AGGTTCACTG	CAACCGTTCA	GAGGTCCCCA	GGAGCTGCTG	CTGGCGAGCC
40	260	270	280		
	CGCTACTGCA	GGGACCTATG	GAGCCATTCC	GTAGTGCCAT	CCCGAGCAAC
	310	320			
45	GCACTGCTGC	AGCTTCCCTG	AGCCTTTCCA	GCAAGTTTGT	TCAAGATTGG
	360	370	380		
	CTGTCAAGAA	TCATGGACTG	; TTATTATATG	CCTTGTTTTC	TGTCAAGACA

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# (1) CC ATG ATT CCT MET Ile Pro

3																		
	GGT Gly	417 AAC ASD	CGA	ATG MET	CIG CIG	ATG MET	432 GIC Val	GTT Val	TTA Læi	TTA Leu	CÀ2 LCC	447 CAA Gln	AST GIC	CTG Leu	CIA Leu	GIY GIY	462 GGC Gly	GCG Ala
10					TIG Leu													
15	522 GGC Gly	CAC His	Ala Ala	GCA Gly	Gly GGA	537 CGC Arg	CGC Arg	TCA Ser	età eee	CAG Gln	552 AGC Ser	CAT His	GAG Glu	CTC Leu	CIG Leu	567 CSG ਮੇੜਬੁ	yzb GYC	TTC Phe
20	GAG Glu	GCC Ala	583 ACI	CIT	cro Leu	CAG Gln	ATG MET	597 TIT Phe	GGG Gly	CIG Leu	CGC Arg	CGC Arg	612 CGC Arg	CCG Pro	CAG Gln	CCT Pro	AGC Ser	627 AAG Lys
25	AGT Ser	GCC Ala	C GIV	C AT	642 C CCG Pro	GAC	TAC Tyr	atg Met	ಸುತ್ತಿ ೧೮೮	657 CAT Asp	CTT	TAC Tyr	ccc Arg	CTT Leu	672 CAG Gln	TCI Ser	GGG Gly	GLU GLU
	GAG Glu	687 GAO Glu	GA)	A GA	GLD GLD	ATC Ile	702 CAC His	AGC Ser	ACI Thir	GGT Gly	CTT Leu	717 GAG Glu	TAT Tyr	CCT	Glu	γπ.d ααο	732	c∞ Ala
30										CAC					CIG			: ATC
<i>35</i>		i GC					TCI					CIC					: AGC	ATC Ile
40				C G					cca					TI				897 GTG Val
						ric					CAC					r TAI		GTT 1 Val
45		G A				_		GI					) AIK					1 YZD 3 GYC 3
50					10 CE					y ccc					r GAI			. Prp
55		G G										A AAG					2. AT	r GAG e Glu

	1122 1137 1152 1167
	CLE YCL CYC CLC CYL CYE YCL CCE YCC CYC CYC CCC CYC CYL CLC YCE YLL YCC
	Val Thr His Leu His Gln Thr Arg Thr His Gln Gly Gln His Val Arg Ile Ser
5	
	1182 1197 1212
	CCA TOS TIA COT CAA COS AST COS AAT TOS COS CAS CITO COS COS CITO CITO GITO
	Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
	1227 1242 1257 1272
10	ACC TIT GGC CAT GAT GGC CGG GGC CAT GCC TIG ACC CGA CGC CGG AGG GCC AAG
	The Pine Gly His Asp Gly Arg Gly His Ala Leu The Arg Arg Arg Arg Ala Lys
	1287 1302 1317
15	CGT AGC CCT ANG CAT CAC TOA CNG CGG GCC AGG NAG NAG NAT NAG NAC TGC CGG
15	Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
	1332 1347 1362 1377
	CCC CAC TOO OTO TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
20	Ary His Ser Leu Tyr Val Asp The Ser Asp Val Gly Trp Asn Asp Trp Ile Val
	1392 1407 1422 1437
	COC COA COA GCC TAC CAG CCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
	Ala Pro Pro Gly Tvr Gln Ala Phe Tvr Cvs His Glv Aso Cvs Pro Phe Pro Leu
25	1452 1467 1482
	COT CAS CAS COT AND AND AND CAT GOT ANT GOT CAS ACC COT GOT ANT TOT
	Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser
	•··· <del>-</del>
30	1497 1512 1527 1542
	GTC AAT TOO AGT ATC COO AAA GOO TGT TGT GTG COO ACT GAA CTG AGT GOO ATC
	Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
	1507
	1557 1572 1587  TOO AIG CIG TAC CIG GAT GAG TAT GAT AAG GIG GIA CIG AAA AAT TAT CAG GAG
35	Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Ash Tyr Gln Glu
	Set wer ten tal ten was did tal was als ten ten and als
	1602 1617 (408) 1636 1645 1656
	ATG GTA GTA GAG GGA TGT GGG TGC GGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
	MET Val Val Glu Gly Cys Gly Cys Arg
40	• • • •
45	
	1666 1676 1686 1696 1706
	ATATACACAC CACACACA CACCACATAC ACCACACACA
50	1716 1726 1736 1746 1756
	1716 1726 1736 1746 1756 TCCACTCACC CACACACTAC ACAGACTGCT TCCTTATAGC TGGACTTTTA
	The state of the s
	1766 1776 1786 1796 1806
55	TTTAAAAAA AAAAAAAAA AATGGAAAAA ATCCCTAAAC ATTCACCTTG
	·

1836

1826

1816

hergestellt wurden, und

Gewinnen des Proteins aus dem Kulturmedium.

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1856

1846

	ACCTTATTTA TGACTTTACG TGCAAATGTT TTGACCATAT TGATCATATA										
5	1866 1876 1886 1896 1906 TTTTGACAAA ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG										
10	1916 1926 1936 1946 AGTCATTATT TTAAAAAAAA AAAAAAAACT CTAGAGTCGA CGGAATTC										
	wobei das Verfahren die nachfolgenden Schritte umfaßt:										
15	(a) Absuchen einer Genbank durch Hybridisieren mit einem markierten bBMP-2-Fragment, wobei die Genbank aus einer von U-2 OS abgeleiteten DNA oder cDNA konstruiert war,										
	(b) Isolieren positiver Clone und										
	(c) Isolieren der DNA-Insertionen aus diesen Clonen.										
20	12. Verfahren nach Anspruch 11, dadurch gekennzeichnet, daß das Gen menschliches BMP-4 codiert, das die in Anspruch 11 angegebene Aminosäuresequenz aufweist.										
25	13. Verfahren zur Herstellung eines Genes, das ein Protein codiert, das Eigenschaften von BMP-4 zeigt, und eine DNA-Sequenz umfaßt, die:										
	(a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 11 unterscheidet;										
	(b) mit einer DNA-Sequenz nach Anspruch 11 oder vorstehendem Absatz (a) hybridisiert; oder										
30	(c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 11 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht,										
	wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt.										
35	14. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist.										
	15. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist.										
40	16. Vektor, enthaltend das Gen oder die DNA-Sequenz nach einem der Ansprüche 1 bis 15 in einer funktionellen Verbindung mit einer Expressions-Kontrollsequenz.										
	17. Zelle, dadurch gekennzeichnet, daß sie mit einem Vektor nach Anspruch 16 transformiert ist.										
45	18. Zelle nach Anspruch 17, dadurch gekennzeichnet, daß sie eine Säugerzelle, eine Bakterienzelle, eine Insektenzelle oder eine Hefezelle ist.										
	19. Zelle nach Anspruch 18, dadurch gekennzeichnet, daß sie eine CHO-Zelle ist.										
50	20. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-2 zeigt, umfassend die Schritte										

21. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-4 zeigt, umfassend die Schritte

Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Expressionsvektor ein Gen oder eine DNA-Sequenz umfaßt, die nach einem der Ansprüche 1 bis 10

- Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Expressionsvektor ein Gen oder eine DNA-Sequenz umfaßt, die nach einem der Ansprüche 11 bis 15 hergestellt wurden, und
- Gewinnen des Proteins aus dem Kulturmedium.
  - 22. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-2 oder BMP-4 zeigt, umfassend die Schritte
    - Züchten der Zelle nach Anspruch 17 in einem geeigneten Kulturmedium und
    - Isolieren des Proteins aus dem Kulturmedium.
  - 23. Verfahren zur Herstellung eines Arzneimittels, dadurch gekennzeichnet, daß es ein Kombinieren der nach einem der Ansprüche 20 bis 22 hergestellten Proteine, einzeln oder in Kombination, mit einem pharmakologisch verträglichen Träger umfaßt.
    - 24. Verfahren nach Anspruch 23, dadurch gekennzeichnet, daß das Arzneimittel femer eine Matrix umfaßt, die fähig ist, das Arzneimittel an die Stelle des Knochen- oder Knorpelschadens zu liefern und eine Struktur zur Induktion der Knochen- oder Knorpelbildung bereitzustellen.
    - 25. Verfahren nach Anspruch 24, dadurch gekennzeichnet, daß die Matrix Hydroxyapatit, Kollagen, Polyessigsäure oder Tricalciúmphosphat umfaßt.
  - **26.** Verwendung eines Proteins nach einem der Ansprüche 20 bis 22, einzeln oder in Kombination, zur Herstellung eines Arzneimittels zur Induktion der Knochen- oder Knorpelbildung.

#### Revendications

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Revendications pour les Etats contractants suivants : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Gène codant pour la BMP-2 humaine comprenant la séquence d'ADN suivante :

	GIV.	CZCT	10	GEOT		20	~~~		0 ~	~~~	40 . بىسى		ra s m	50	C3.CC		60	AACIT	70
5			C.L.13	CALL	G1G1	G1 (	ملحم	-110	G CI	993	VCTI	CII	GW.	.10	CM3G	onun.	<b>.</b>	WWCT I	محدد
	œ	ACT	80 TTG	<u> </u>		90 Œ I	TIGO	10 XXX		GAGO	CIGC		_	20 CT	ಯಾಸಿ		30 CA (	 ന് <del>ട</del> മ	140 CCTC
10	ACD		150 GGC	CITG		60 AC A	.CTCA	17 EXCG	-	TTCC	180 CAGC			.90 .26	AGAC		00 CG (	cocc	CXCCC 210
15	GGG		220 GGA	GGÀG		30 AG A	AA}G	24 GAAO		CATT	250 CGGT			:60 :CA	GGIC		70 GA (	, ) )	280 GITI
20	TCC		290 GGA	ಹದಾ		00. CA A	TGCZ)	.31: OGIG			320 GTGC		_	30 .cs	GACI		40 IC 1	rcci»	:350 350
25	_CCE4(	$\infty$ $\lambda$				SS A					ea G		rg CI			$\infty$ $^{\circ}$			
	CTC Leu	CIG Leu	ejà ecc	415 GGC Gly	CCC SLA	CCT Ala	GJ. GGC	CIC CIC	430 CIT Val	003 Px10	GAS Glu	CIG Leu	GGC	445 CGC Arg	AGG	TÀ2 YYC	TIC Phe	s YTs c ccc	
30	450 GCG Ala	GŒ Ala	TCS Ser	TCG Ser	GGC	475 CCC Arg	$\infty$	TCA Ser	TCC Ser	C\G Gln	490 220	TCT Sex	y <del>2</del> 5 ( С7С (	C}G Glu	GTC Val	505 CIG Leu	ACC Ser	Glu	
35	TIC Phe	GłG Glu	520 TTG Leu	೦೦೦	CTG Leu	Ten CLC	AGC Ser	535 ATG FET	TTC Phe	GGC Gly	CTG Leu	AAA Lys	550 CAG . Glm .	AGA Azg	223	ACC Thr	27.C	565 2. XSC 5 Ser	
40																		ejà el	
																		o A GCC A Ala	
45	AAC Asn	ACT Thr	متو متو	685 CSC Arg	AGC Ser	TTC Phe	CAC His	CAT CAT	700 GAA Glu	GJU G7Y	TCT Ser	TTG Leu	G2A	715 GAA Glu	CIA	CCY Sto	ردی Glv	TITE A ACC	

	730					745					760					775		
	ACT	GGG	AAA	ACA	$\infty$ A	$\alpha$	AGA	TTC	TTC	TTT	TAA	TTA	agt	TCI	ATC	$\infty$	ACG	GYG
5	Ser	Gly	ŢÃ2	Thr	<u> </u>	भ्रापु	Arg	Phe	Phe	Phe	Asn	Leu	Ser	Ser	Ile	220	The	Glu
			790			•		805					820					835
	GAG	TIT		AΦ	TCA	GCA	GAG		CAG	GIT	TTC	ŒÀ		CAG	λIG	CAA	GAT	
				Thr														
10																		
	ميس	CC \	330	TAA	850	ىسىد	<u></u>	C3 C3	<b>~</b>	865	31111	יחממ	2011	സസ	088	איזיי	בידב	332
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15		895					910					925					940	
				GCC														
	PIO	ALA	unr	Ala	ASN	Ser	TÀZ	me	PTO	VZI	um	Ser	Leu	Ten	vzĎ	1111	Ary	TELL
				955					970					985				
20	GIG	λλŢ	CAG	AAT	GCA	AGC	AGG	TGG	GYY	AGT	TIT	GAT	GIC	ACC	$\infty$	GCT	GIG	ATG
	Val	yzu	Gln	Asn	Ala	Ser	Arg	đđ	Glu	Ser	Phe	yzb	Val	Thr	Pro	Ala	Val	MET
	1000	3			-	1015				-	1030				7	1045		
		-	ACT	GCA			CAC	GΩ	AAC			TIC	GIG	GTG	_		GΩ	CAC
25	Arg	Trp	Thr	Ala	Gln	Gly	His	Ala	ÀSN	His	Gly	Phe	Val	Val	Glu	Val	Ala	His
23	_					<b>-</b> .					-							
			1060					1075					1090		360	366	-	1105
,				AAA Lys														
30		صنط	GIU	. LL	GIII	Gry	۷۵.	عد	Lys	Αrg	حدد	٧	.a.g			,,,,		
					1120					1135					1150			
				GAA														
	ars	GLn	yzb	Glu	His	Ser	Trp	Ser	Gln	Ile	Arg	Pro	Leu	Leu	Val	mr	∴ne	GTÅ
35		1155				•	1180					1195				:	1210	
33	CAT	GAT	GGA	AAA	GGG	CAT	$\alpha$	CIC	CAC	AÁA	AGA	GAA	AAA	$\alpha$	$C\lambda\lambda$	$\epsilon \infty$	$\lambda\lambda\lambda$	CAC
	His	∀ಪ	Gly	Lys	Gly	His	Pro	Leu	His	Lys	Arg	Glu	Lys	yrd	Gln	Ala	ŢÑZ	His
			-	1225					1240				-	1255				
40	AAA	CAG		AAA	æ	ىلىل	226			ידאר	אַגג	AGA	-		TTG	TAC	GTG	GAC
40	Lys	Gln	Arq	Lys	Arq	Leu	Lys	Ser	Ser	Cys	Lys	Arg	His	Pro	Leu	Tyr	Val	۸sp
			-	-			-											
	1270					1285					1300		~~	~~		1315	C) C	~~
4=	The	التكثر حدد	GAC	GTG Val	GGG	TGG	AAT.	2.C	766	ATT	Ual	312	2	محر	Gly	LAT.	wic .	21a
45		J=_	لتحت	٧ڪـــ	GTĀ	يين	انحم	ترجم	لوخن	115	٧	Aic	1.0		GLY	-7-		
			1330					1345					1350					1375
	TTT	TAC	TGC	CAC	GGA	GYY	TGC	$\alpha$	TIT	$\alpha$	CIG	GCT	C-T	CAT	CIG	AAC	ICC	ACT
	Phe	$\mathbb{T}^{N_{\perp}}$	CÀ2	His	Gly	Glu	Čž	Pro	Phe	مترة	ĭeu	Ala	تحز	<u>Fi</u> s	Leu	מצל	Ser	777
50				1	1390					1405				,	1420			
	AAT	C-II	GCT.	ATT		Cac	àCT:	عكسك			TCT	GIT	AAC			ATT	$\alpha$	AAG
	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	אבה	Ser	Val	Asn	Ser	Lys	Ile	Pro	Lys
							_	_							-			

		$\cdot$
		1435 1450 1465 1480
		CCA TCC TGT GTC CCG ACA CAA CTC AGT CCT ATC TCG ATG CTG TAC CTT GAC GAG
5		Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu
		1495 1510 1 <i>5</i> 25
		AAT CAA AAG GIT GIA TIA AAG AAC TAT CAG GAC AIG GIT GIG GAG GGT TGT GGG
		Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly
10		1540(396) 1553 1563 1573 1583 1593 1603
		TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA Cys Arg
		cys arg
		••••
15		AAAA
	2.	Gène codant pour la BMP-2 humaine comportant la séquence d'acides aminés donnée à la revendication 1.
	3.	Gène codant pour une protéine montrant des propriétés de la BMP-2 humaine et comprenant une séquence
20		d'ADN :
		(a) qui diffère d'une séquence d'ADN de la revendication 1 dans la séquence de codons du fait de la dégé-
		nérescence du code génétique ;
		(b) qui s'hybride avec une séquence d'ADN de la revendication 1 ou du paragraphe (a) ci-dessus ; ou
25		(c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 1, que cette variation résulte de changements dans la séquence peptidique ou non.
		conte tanado reconte de crangomento dano la coquente popularido de non.
	4.	Séquence d'ADN suivant la revendication 3, qui est une séquence d'ADN génomique.
30	5.	Séquence d'ADN suivant la revendication 3, qui est une séquence d'ADNc.
		, <b>4</b>
	6.	Gène codant pour la BMP-2 bovine comprenant la séquence d'ADN suivante :
35		
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5	(1) GGC G	CAC	GAT D	GGG	15 AAA K	GGA	CAC H	CCT P	CTC L	30 CAC H	AGA R	AGA R			45 CGG R
10	CAA Q	GCA A	AAA K	CAC H	60 AAA K	CAG Q	CGG R	AAA K	CGC R	75 CTC L	AAG K	TCC S	AGC S	TGT C	90 AAG K
	AGA R	CAC H	CCT P	TTA L	105 TAT Y	GTG V	GAC D	TTC F	AGT S	120 GAT D	GTG V	GGG G	TGG W	AAT N	135 GAC D
15	TGG W	ATC I	GTT V	GCA A	150 CCG P	CCG	GGG G	TAT Y	CAT H	165 GCC A	TTT F	TAC Y	TGC C	CAT H	180 GGG G
20	GAG E	TGC	CCT P	TTT F	195 CCC P	CTG L	GCC A	GAT D	CAC H	210 CTT L	AAC N	TCC S	ACG T	AAT N	225 CAT H
	GCC.	ATT	CTC.	CAA Q	240 ACT T	CTG: L	GTC V	AAC N	TCA S	255 GTT V	AAC N	TCT	'AAG K	ATT I	270 CCC P
30	AAG	GCA A	TGC	TGT	GTC	CCA	ACA	GAG	CTC L	AGC	GCC	ATC I	TCC S	ATG M	315 CTG L
	TAC Y	CTT L	GAT D	GAG E	330 AAT N	GAG E	AAG K	GTG V	GTA V	345 TTA L	AAG K	AAC N		CAG O	360 GAC
35	ATG M	GTT V	GTC V	GAG E	GGT	TGT C	GGG	TGT	(129 CGT R	)) TAGO	CACAC	97 CA A	ATA	40 LAAAT	7 'A
40	TAAA	4 TATA	17 .TA T	'ATAT	42 ATAT	7 'A TT	'AGAA	437 AAAC	AGC	AAAA	447 AAA	TCAA	4 GTTG	57 AC	
45	ACTI		67 AT T	TCCC	47 AATG		ACTT	487 TATT	TAT	'GGAA	497 TGG	AATG	5 GAGA	07 AA	
	AAGA		17 CA C	AGCT	52 ATTT	7 T GA	AAAC	537 TATA	TTT	'ATAT	547 CTA	CCGA	5 AAAG	57 AA	
50	GTTG		67 AA C	aaat	57 ATTT		TCAG	587 AGAA	TTA	TT					

- 7. Gène codant pour la BMP-2 bovine contenant la séquence d'acides aminés de la revendication 6.
- 8. Gène codant pour une protéine montrant des propriétés de la BMP-2 bovine et comprenant des séquences d'ADN :

(a) qui diffèrent d'une séquence d'ADN de la revendication 7 dans la séquence des codons du fait de la dégénérescence du code génétique ;

- (b) qui s'hybrident avec une séquence d'ADN de la revendication 7 ou du paragraphe (a) ci-dessus ; ou (c) représentent des fragments, des variations alléliques ou autres d'une séquence d'ADN de la revendication
- 7, que ces variations résultent de changements dans la séquence peptidique ou non.
- 9. Séquence d'ADN suivant la revendication 8, qui est une séquence d'ADN génomique.
  - 10. Séquence d'ADN suivant la revendication 8, qui est une séquence d'ADNc.

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11. Gène codant pour la BMP-4 humaine comprenant la séquence d'ADN suivante :										
70	10 CTCTAGAGGG	20 CACAGGAGGA	0E <b>2342334223</b>	40 CAAGGAGCCCC	50 GGAGOCOGGC	CCCCGFFCCIF 60	70 GGTGAGTGTG			
15	08 <b>2242222</b> 429	90 T <del>CACCGACC</del> C	100 GAGOCIGAGA	110 CCCCCCCT	120 GCTCCGGCTG	130 AGTATCEAGC	140 TTGTCTCCCC			
20	150 CATCSGATTC	160 COGTOCAAGC	170 TATCIOSAGE	180 CTGCAGGGGC	190 ACAGTCCCCG	200 GCCCTCGCCC	210 AGGITCACIG			
	220 CAACCETTCA	C+CC.100007	240 GGAECTGCTG		260 CECTACTECA	270 GGGACCTATG	280 GASCCATTCC			
25	290 GTAGTGCCAT				330 AGCCTTTCCA		350 TCAAGATTGG			
30	360 CTGTCAAGAA	370 TCATGGACTG	380 TIATIATATG	390 CTTGTTTTC	400 TGTCAAGACA		ccr			

MET Tle Pro

5	GGT :	417 AAC Asn	Yrg CCY	ATG MET	CTG Leu	atg Met	432 GIC Val	GIT Val	TTA Leu	TEA Leu	TGC Cys	447 CAA Gln	GTC Val	CTG Leu	CTA Leu	GGA Gly	462 GGC Gly	GCG Ala
																		CAG
10	522 GGC Gly	CAC His	GCG Ala	GGA Gly	GJA GGY	537 ŒC Arg	OGC Arg	TCA Ser	GGG Gly	Gln Cag	552 AGC Ser	CAT His	GAG Glu	CIC Leu	CIG Leu	567 ŒG Arg	GAC Asp	TTC Phe
15	GAG (																	
20	AGT (													CIT Leu				
																		GCC Ala
25	AGC Ser	CGG Arg	GCC Ala	747 AAC Asn	ACC	GTG Val	AGG Ara	AGC Ser	762 TTC Phe	CAC	CAC His	GAA Glu	GAA Glu	777 CAT His	CTG -Leu	GAG -Glu	AAC Asn	ATC
<b>30</b>	792 ∝A	ccc	ACC	AGT	GAA.	807 AAC	TCT	GCT	TTT	CI	822 TTC	CIC	TTT		CTC	837 AGC	AGC	ATC
35	CCT Pro	GJu GJu	852 AAC Asn	GAG	GIG Val	ATC Lle	TCC Ser	867 TCT Ser	GCA Ala	G}n GAG	CIT Leu	OGG Arg	882 CTC Leu	TTC Phe	CGG Arg	GAG Glu	CAG Gln	897 GTG Val
. 40	GAC Asp	CAG Gln	GGC Gly	CCT Pro	912 GAT Asp	Trp TGG	GAA Glu	Acc Acc	GGC Gly	927 TTC Phe	CAC	OCI	ATA Ile	AAC Asn	942 ATT Ile	TAT Tyr	GAG Glu	GIT Val
	atg Met	957 AAG Lys	$\infty$	CCA Pro	GCA Ala	€£X	972 GIG Val	GIG Val	CCT Pro	GGG Gly	CAC His	987 CTC Leu	ATC	ACA Thr	OGA Arg	CIA	1002 CTG Leu	yzb GyC
45	ACG Thr	ycy ycy	CIG	1017 GIC Val	CAC His	C-C Fis	AAT A <u>Ş</u>	GTG	1032 ACA Thr	CGG Arg	TITE TGG	GAA Glu	ACT	1047 TTT Phe	C÷T Ç÷Ç	CIG Val	AGC Ser	CCT CCT
50	1062 GCG	GIC	CTT	œc	TCC	1077 ACC	೦೦೦೦	ಎ	عدد	್ಷಲ್ಟ	1092 CCA	AAC	TAT		CIA	1107 G∝	. ATT	GAG .
55	GIG Val	ACT	1122 CAC His	CIC Leu	CAT His	eju Ge	ACT	1137 Arg Arg	ACC Thr	CAC His	CAG Gln	GGC	1152 CAG Gln	CAT His	GTC Val	AGG Arg	ATT	1167 AGC Ser

5	1162 1197 1212  CGA TCC TTA CCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTG GTC  Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
J	1227 1242 1257 1272 ACC TIT GGC CAT GAC GGG GGC CAT GCC TIG ACC CGA GGC GGC AAG Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys
10	1302 1317 CCT AGC CCT AAG CAT CAC TCA CAG CCC AGG AAG AAG AAT AAG AAC TGC CCG Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
15	1332 1347 1362 1377 CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
20	1392 1407 1422 1437 GCC CCA CCA GGC TAC CCAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG Ala Pro Pro Glv Tvr Gln Ala Phe Tvr Cvs His Glv Aso Cvs Pro Phe Pro Leu
	1452 1467 1482 GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser
25	1497 1512 1527 1542 GIC AAT TOO AGT ATO COO AAA GOO TOT TOT GIG COO ACT GAA CIG AGT GOO ATO Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
30	1557 1572 1587 TCC ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
35	1602 1617 (408) 1636 1646 1656 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG MET Val Val Glu Gly Cys Gly Cys Arg
	1666 1676 1686 1695 1706 1716 1726 ATATACACAC CACACACAC CACCACATAC ACCACACAC CACGACTCCCA TOCACTCACC CACACACTAC
40	1736 1746 1756 1766 1776 1786 1796 ACAGACIGCT TOCTTATAGC TEGACITITA TITAAAAAA AAAAAAAAA AATEGAAAAA ATCCCTAAAC
45	1806 1816 1826 1836 1846 1855 1866 ATTCACCITG ACCITATITA TEACHTEACG TECANATEST THEACCATAT TEATCATATA THITEACAAA
50	1876 1886 1895 1906 1916 1925 1935 ATATATTTAT AACTACSTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAAA AAAAAAAACT
	1946 CTAGAGTOGA CGGAATTC
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- 12. Gène codant pour la BMP-4 humaine comportant la séquence d'acides aminés donnée à la revendication 11.
- 13. Gène codant pour une protéine montrant des propriétés de la BMP-4 et comprenant une séquence d'ADN :

- (a) qui diffère d'une séquence d'ADN de la revendication 11 dans la séquence des codons du fait de la dégénérescence du code génétique ;
- (b) qui s'hybride avec une séquence d'ADN de la revendication 11 ou du paragraphe (a) ci-dessus ; ou
- (c) représente un fragment; une variation allélique ou autre d'une séquence d'ADN de la revendication 11, que cette variation résulte de changements dans la séquence peptidique ou non.
- 14. Séquence d'ADN suivant la revendication 13, qui est une séquence d'ADN génomique.
- 15. Séquence d'ADN suivant la revendication 13, qui est une séquence d'ADNc.
- 16. Vecteur contenant le gène ou la séquence d'ADN suivant l'une quelconque des revendications 1 à 15, en association active avec une séquence de contrôle d'expression.
- 17. Cellule transformée avec un vecteur de la revendication 16.

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- 18. Cellule suivant la revendication 17, qui est une cellule mammifère, une cellule bactérienne, une cellule d'insecte ou une cellule de levure.
- 19. Cellule suivant la revendication 18, qui est une cellule CHO.
- 20. Protéine montrant des propriétés de la BMP-2, qui est codée par un gène ou une séquence d'ADN de l'une quelconque des revendications 1 à 10.
- 21. Protéine montrant des propriétés de la BMP-2, qui est obtenable par les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN de l'une quelconque des revendications 1 à 10, et de récupération de ladite protéine du milieu de culture précité.
  - 22. Protéine montrant des propriétés de la BMP-4, qui est codée par un gène ou une séquence d'ADN de l'une quelconque des revendications 11 à 15.
  - 23. Protéine montrant des propriétés de la BMP-4, qui est obtenable par les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN de l'une quelconque des revendications 11 à 15, et de récupération de ladite protéine du milieu de culture précité.
  - 24. Procédé de production de la protéine suivant l'une ou l'autre des revendications 21 et 23, comprenant les étapes de culture dans un milieu de culture approprié de la cellule de la revendication 17 et d'isolement de ladite protéine du milieu de culture précité.
    - 25. Composition pharmaceutique comprenant les protéines de l'une quelconque des revendications 20 à 23, individuellement ou en combinaison, et un véhicule pharmaceutiquement acceptable.
      - 26. Composition pharmaceutique suivant la revendication 25, comprenant de plus une matrice pouvant distribuer la composition au site de l'anomalie osseuse ou cartilagineuse et formant une structure pour induire une formation osseuse ou cartilagineuse.
      - 27. Composition pharmaceutique suivant la revendication 26, dans laquelle ladite matrice comprend de l'hydroxyapatite, du collagène, de l'acide polylactique ou du phosphate tricalcique.
- 28. Utilisation d'une protéine suivant l'une quelconque des revendications 20 à 23, individuellement ou en combinaison, pour la préparation d'une composition pharmaceutique pour induire une formation osseuse ou cartilagineuse.

#### Revendications pour l'Etat contractant suivant : AT

55 1. Procédé de préparation d'un gène codant pour la BMP-2 humaine comprenant la séquence d'ADN suivante :

	10 GTCGACTCTA	eyelelelel Cy 50	30 ECACITEG CTEGGG	.40 50 ACIT CTIGAACITG	60 70 CAGGGAGAAT AACITGGGCA
5	08 OCCACTITIC	90 CCCCCCCC TT	100 TGCCCCAG CCGAGC	110 120 CTGC TTGGCCATCT	130 140 COCACOOOXA COCCOOXICC
10	150 ACTOCTOGGC	160 CTTGCCCCAC AC	170 TGAGACSC TGTTCC	180 190 CAGC GTGAAAAGAG	200 210 AGACTGOGGG GCGGGGACCC
15	220 GGGAGAAGGA	230 GGAGGCAAAG AA	240 AACCAACG GACATT	250 260 OGGT OCTTGCGCCA	270 280 GGTOCTITGA CCACAGITIT
20	29.0 TOCATGTGGA	300. CCCTCTTTCA AT		320 330 STEC TICTIPEACS	340 350 GACTGCGGTC TCCTAAACGT
ar.	(1) CEACC ATG ( MET )	37 FTG GOO GGG AO Val Ala Gly Th	c coc ref crr c	385 FA GOS TTG CTG BU Ala Leu Leu	400 CIT CCC CAG GIC Leu Pro Gln Val
25					5 C AGG AAG TTC GCG g Arg Iys Phe Alz
30					505 G GTC CTG AGC GAG u Val Ieu Ser Glu
35		cos crs crc . 1 Arg Leu Leu	Ser MET Phe Gly		565 A CCC ACC CCC ACC g Pro Thr Pro Ser
40	ಳಾಡಿ y <del>2</del> ರ y1	a Val Val Pro	Pro Tyr MET Leu	Asp Leu Tyr Ar	610 C ACG CAC TCG GGT g Arg His Ser Gly
	625 CAG CCG GG GLA PRO GL	o TCA CCC GCC y Ser Pro Ala	Pro Asp His Arg	Ieu Glu Arg Al	670 A GCC AGC CGA GCC a Ala Ser Arg Ala
45	AAC ACT GT Asn Thr Va	685 G CCC AGC TIC L Arg Ser Phe	700 CAC CAT GAA GAA His His Glu Glu	71 TCT TTG GAA GA Ser Leu Glu Gl	5 A CTA CCA GAA ACG u Leu Pro Glu Thr

5	730 AGT GGG Ser Gly	AAA ACA Lys Thr	74: ACC CGC Thr Arc	AGA TIY	C TIC TII B Phe Phe	760 AAT TIA ASI ASn Leu Sei	775 T TCT ATC CCC ACG GAG T Ser Ile Pro Thr Glu
v	GAG TTT Glu Phe	790 ATC ACC Ile Thr	TCA GC: Ser Ala	805 A GAG CIT Clu Leu	CAG GIT	820 TTC CGA GAA Phe Arg Glu	835 CAG ATG CAA GAT GCT Gln MET Gln Asp Ala
10	TTA GGA Leu Gly	AAC AAT Asn Asn	850 AGC AGI Ser Ser	TIC CAI	865 CAC CEA His Arg	TA TAK TIK	880 TAT GAA ATC ATA AAA Tyr Glu lle lle lys
15	895 CCT GCA Pro Ala	ACA GCC	AAC TOO Asn Ser	910 AAA TIC Lys Phe	c ccc crc Pro Val	925 ACC AGT CIT Thr Ser Lev	940 TITG GAC ACC AGG TIG Leu Asp Thr Arg Leu
20	GTG AAT Val Asn	955 CAG AAT Gln Asn	GCA AGO	AGG TGG	970 GAA AGT Glu Ser	TTT GAT GTO Phe Asp Val	985 ACC CCC GCT GTG ATG Thr Pro Ala Val MET
	yrd lib Cee lee yrd lib	ACT GCA Thr Ala	1015 CAG GGA Gln Gly	CAC GCC	AAC CAT	1030 GGA TTC GTG Gly Phe Val	1045 GIG GAA GIG GCC CAC Val Glu Val Ala His
25	TTG CAG	GJU IYS GAG AAA 1060	CAA GGI Gln Gly	1075 GIC TO Val Ser	: AAG AGA	1090 CAT GIT AGG His Val Airg	1105 ATA AGC AGG TCT TTG Ile Ser Arg Ser Leu
30	CAC CAA His Gln	GAT GAA	ll20 CAC AGO His Ser	TGG TCA	ll35 CAG ATA Gln Ile	AGG CCA TIG Arg Pro Leu	1150 CTA GTA ACT TTT GGC Leu Val Thr Fne Gly
35	ll65 CAT GAT His Asp	GGA AAA Gly Lys	GGG CAT	1180 CCT CTC Pro Len	CAC AÁA His Lys	1195 AGA GAA AAA Arg Glu Lys	1210 CCT CAA CCC AAA CAC Arg Gln Ala Lys His
	AAA CAG Lys Gln	1225 CG AAA Arg Lys	CGC CTT Arg Leu	AAG TCC	1240 AGC TGT Ser Cys	AAG AGA CAC	1255 CCT TTG TAC GTG GAC Pro Leu Tyr Val Asp
40	1270 TTC AGT Phe Ser	yeb Asj GyC QIC	1285 <i>حم</i> د عص وريل و12	YZI GYC CYC	TGG ATT	300 GTG GCT CCC Val Ala Pro	1315 CCG GGG TAT CAC GCC Pro Gly Tyr His Ala
45	TIT TAC	330 TGC CAC Cys His	GGA GAA Gly Glu	1345 TGC CCT Cys Pro	TTT CCT	1350 CTG GCT GAT Leu Ala Asp	1375 CAT CTG AAC TCC ACT His Leu Asn Ser Thr
<i>50</i>	AAT CAT ( Asn His )	GCC ATT	390 CTT CAG Val Gln	ACG TTG	1405 GTC AAC Val Asn	TCT GTT AAC Ser Val Asn	1420 TOT AMS ATT COT AMS Ser Lys Ile Pro Lys

1435 1450 1465 1480 GCA TGC TGT GTC COG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu 5 1495 1525 1510 AAT GAA AAG GIT GIA TIA AAG AAC TAT CAG GAC AIG GIT GIG GAG GGI TGI GGG Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly 10 1540 (396) 1553 1563 1573 1583 1603 TGT CSC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTITTAG AAAAAAGAAA Cys Arg 15 AAAA , dans lequel ledit procédé comprend les étapes suivantes a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant de U-2 OS avec 20 un fragment de bBMP-2 marqué par hybridation, b) l'isolement des clones positifs, et c) l'isolement des inserts d'ADN de ces clones. 2. Procédé suivant la revendication 1, dans lequel le gène code pour la BMP-2 humaine ayant la séquence d'acides: 25 aminés donnée à la revendication 1. 3. Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-2 humaine et comprenant une séquence d'ADN: 30 a) qui diffère d'une séquence d'ADN de la revendication 1 dans la séquence des codons du fait de la dégénérescence du code génétique; b) qui s'hybride avec une séquence d'ADN de la revendication 1 ou du paragraphe (a) ci-dessus ; ou c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 1, que cette variation résulte de changements dans la séquence peptidique ou non, 35

dans lequel le procédé susdit comprend des techniques standards de biologie moléculaire.

- 4. Procédé suivant la revendication 3, dans lequel la séquence d'ADN est une séquence d'ADN génomique.
- 5. Procédé suivant la revendication 3, dans lequel la séquence d'ADN est une séquence d'ADNc.
  - 6. Procédé de préparation d'un gène codant pour la BMP-2 bovine comprenant la séquence d'ADN suivante :

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      GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG
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      CAA GCA AAA CAC AAA CAG CGG AAA CGC CTC AAG TCC AGC TGT AAG
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                                                 S
                 H
                     K
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                             R
                                 K
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                                         120
                      105
      AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC
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                      150
      TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT TAC TGC CAT
15
                                                             GGG
                                     Н
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      GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT
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                      240
      GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT CCC
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      AAG GCA TGC TGT GTC CCA ACA GAG CTC AGC GCC ATC TCC ATG CTG
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                              T
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                                          S
                                              A
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                                          345
                      330
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         ACTITAATAT TTCCCAATGA AGACTITATT TATGGAATGG AATGGAGAAA
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                           527
                                       537
                                                  547
         AAGAAAACA CAGCTATTTT GAAAACTATA TTTATATCTA CCGAAAAGAA
                567
                           577
                                       587
         GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT,
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dans lequel ledit procédé comprend les étapes suivantes :

a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant de foie bovin avec une sonde marquée conçue sur la base de la séquence d'acides aminés d'un fragment de bBMP-2,

b) l'isolement des clones positifs, et

c) l'isolement des inserts d'ADN de ces clones.

<sup>7.</sup> Procédé suivant la revendication 6, dans lequel le gène code pour de la BMP-2 bovine ayant la séquence d'acides

		aminés de la revendication 6.
5	8.	Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-2 bovine et comprenant des séquences d'ADN :
·		<ul> <li>a) qui diffèrent d'une séquence d'ADN de la revendication 7 dans la séquence des codons du fait de la dégénérescence du code génétique;</li> </ul>
10		<ul> <li>b) qui s'hybrident avec une séquence d'ADN de la revendication 7 ou du paragraphe a) ci-dessus ; ou</li> <li>c) représentent des fragments, des variations alléliques ou autres d'une séquence d'ADN de la revendication</li> <li>7, que ces variations résultent de changements dans la séquence peptidique ou non,</li> </ul>
		dans lequel le procédé précité comprend des techniques standards de biologie moléculaire.
15	9.	Procédé suivant la revendication 8, dans lequel la séquence d'ADN est une séquence d'ADN génomique.
	10.	Procédé suivant la revendication 8, dans lequel la séquence d'ADN est une séquence d'ADNc.
	11.	Procédé de préparation d'un gène codant pour la BMP-4 humaine comprenant la séquence d'ADN suivante :
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	10 CTCTAGAGGG	20 CAGAGGAGGA	GCCACCCACC		50 CCACCCCCCC	ccccyycciy e0	70 GGTGAGTGTG
5	08 CCATOOGACO	90 TGAGGGAGGC	100 GAGOCTGAGA	110		_	140 TTGTCTCCCC
10	150 GATGGGATTC	160 CCSTCCAAGC	170 TATCICGAGC	180 CIGCAGCGCC		200 CCCTCCCC	210 AGGITCACIG
15	220 CAACOCITCA	230 CAGTTOOTA	240 GGAGCTGCTG	250 CTGGCGAGCC	260 CCTACTCCA		280 GAGCCATTCC
	290 GIAGIGCCAT	300	310 GÇACTGCTGC	320 ACCTICCCTG	330 ACCUTITODA		
20	360 CTGTCAAGAA	370 TCATCCACTG		390 CETTGITTIC	400 TGTCAAGACA		
25						121,124	
30	417 GGT AAC ( Gly Asn A	TEA ATG CTG	432 ATG GTC GTI MET Val Val	TTA TEA TO Leu Leu Cy	447 SC CAA CTC C rs Gln Val I	TTG CTA GGA Leu Leu Gly	462 GGC GCG Gly Ala
	AGC CAT ( Ser His A	477 SCT AGT TIG Ala Ser Leu	ATA CCT GAO Ile Pro Glu	492 ACG GGG AA Thir Gly Ly	C AAA AAA O	507 TC GCC GAG /al Ala Glu	ATT CAG Ile Gln
35	522 GGC CAC ( Gly His ?	rja ejh ejh ece ech ech	537 CCC CCC TCR Arg Arg Ser	55 A GGG CAG AG Gly Gln Se	C CAT CAG (	557 CTC CTG CCG Leu Leu Arg	CAC TTC Asp Phe
40	ಚಿತ್ರ ತಿಂದ್ರ	582 ACA CIT CIG Thr Leu Leu	597 CAS ATG TIT Gln MET Phe	ന്ദ്രേയ അവ	ed yrd yrd i ec cec cec ( e13	oce cye cci cce cye cci	627 AGC AAG Ser Lys
45	AGT GCC ( Ser Ala V	642 FIC ATT COS /al Ile Pro	CAC TAC ATO Asp Tyr MEI	657 CGG GAT CI Arg Asp Le	TT TAC CGG ( au Tyr Arg 1	672 TIT CAG TCT Leu Gln Ser	ece ere
	687 GAG GAG ( Glu Glu (	EAA GAG CAG Slu Glu Gln	702 ATC CAC AG Ile His Ser	ACT GGT CI	717 TT CAG TAT ( eu Glu Tyr I	CCT GAG CGC Pro Glu Arg	732 CCG GCC Pro Ala
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																	AAC	
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30	GIG Val	ACT The	CAC	CIC	CAT	CAG	ACT	œ	λCC	CAC	CYC	GGC	CXC	CAT	GIC.	AGG	ATT.	AGC
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55	וב	a As	iH G	s La	عم ب	n Se		I AS	n m	SAL	للت	e va	T GT	n $m$	عد عد	L VC		n Ser

GTC AAT TOO AGT ATC COO AAA GOO TGT TGT GTG COO ACT GAA CTG AGT GOO ATC Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile TOO ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu (408)ATE GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG MET Val Val Glu Gly Cys Gly Cys Arg ATATACACAC CACACACACA CACCACATAC ACCACACA CACCTTOCCA TOCACTCACC CACACACTAC ACAGACTECT TCCTTATAGC TEGACTITTA TITAAAAAA AAAAAAAAA AATEGAAAAA ATCCCTAAAC ATTCACCITE ACCITATELA TEACHTEAG TECANALIGIT TICACCATAT TEATCATATA TITTEACANA: ATATATTTAT AACTACGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TEXAAAAAA AAAAAAACT CTAGAGTOGA CGGAATTC, dans lequel le procédé précité comprend les étapes suivantes : a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant d'U-2 OS avec un fragment bBMP-2 marqué par hybridation, b) l'isolement des clones positifs, et c) l'isolement des inserts d'ADN de ces clones. 12. Procédé suivant la revendication 11, dans lequel le gène code pour la BMP-4 humaine ayant la séquence d'acides aminés donnée à la revendication 11. 13. Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-4 et comprenant une séquence d'ADN: a) qui diffère d'une séquence d'ADN de la revendication 11 dans la séquence des codons du fait de la dégénérescence du code génétique :

dans lequel le procédé précité comprend des techniques standards de biologie moléculaire.

cette variation résulte de changements dans la séquence peptidique ou non,

- 14. Procédé suivant la revendication 13, dans lequel la séquence d'ADN est une séquence d'ADN génomique.
- 15. Procédé suivant la revendication 13, dans lequel la séquence d'ADN est une séquence d'ADNc.
- 16. Vecteur contenant le gène ou la séquence d'ADN préparé suivant l'une quelconque des revendications 1 à 15, en

b) qui s'hybride avec une séquence d'ADN de la revendication 11 ou du paragraphe a) ci-dessus ; ou c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 11, que

association active avec une séquence de contrôle d'expression.

- 17. Cellule transformée avec un vecteur de la revendication 16.
- 5 18. Cellule suivant la revendication 17, qui est une cellule mammifère, une cellule bactérienne, une cellule d'insecte ou une cellule de levure.
  - 19. Cellule suivant la revendication 18, qui est une cellule CHO.
- 20. Procédé de préparation d'une protéine montrant des propriétés de la BMP-2, dans lequel ledit procédé comprend les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN préparé suivant l'une quelconque des revendications 1 à 10 et de récupération de ladite protéine du milieu de culture précité.
- 21. Procédé de préparation d'une protéine montrant des propriétés de la BMP-4, dans lequel ledit procédé comprend les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN préparé suivant l'une quelconque des revendications 11 à 15 et de récupération de ladite protéine du milieu de culture précité.
- 20 22. Procédé de production d'une protéine montrant des propriétés de la BMP-2 ou BMP-4, comprenant les étapes de culture dans un milieu de culture approprié de la cellule de la revendication 17 et d'isolement de ladite protéine du milieu de culture précité.
  - 23. Procédé de préparation d'une composition pharmaceutique comprenant la combinaison des protéines préparées suivant l'une quelconque des revendications 20 à 22, individuellement ou en combinaison avec un véhicule pharmaceutiquement acceptable.
    - 24. Procédé suivant la revendication 23, dans lequel la composition pharmaceutique susdite comprend de plus une matrice pouvant distribuer la composition au site de l'anomalie osseuse ou cartilagineuse et constituer une structure pour induire une formation osseuse ou cartilagineuse.
    - 25. Procédé suivant la revendication 24, dans lequel la matrice comprend de l'hydroxyapatite, du collagène, de l'acide polylactique ou du phosphate tricalcique.
- 26. Utilisation d'une protéine préparée suivant l'une quelconque des revendications 20 à 22, individuellement ou en combinaison, pour la préparation d'une composition pharmaceutique pour induire une formation osseuse ou cartilagineuse.

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